



HANDBOOK OF

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Media for  
Environmental  
Microbiology

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SECOND EDITION

By

RONALD M. ATLAS

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RONALD M. ATLAS



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**About the Author:**

Ronald M. Atlas, Ph.D., is dean of the Graduate School, professor of Biology, professor of Public Health, and codirector of the Center for the Deterrence of Biowarfare and Bioterrorism at the University of Louisville. He received his B.S. from the State University of New York at Stony Brook in 1968 and his M.S. and Ph.D. from Rutgers in 1970 and 1972, respectively. After spending a year at the Jet Propulsion Laboratory in Pasadena, California, he joined the faculty at the University of Louisville. He also has served as an Adjunct Professor at the University of Puerto Rico, External Examiner at the National University of Singapore, External Examiner at the Chinese University in Hong Kong, and Extraordinary Professor of Microbiology at the University of Pretoria. Dr. Atlas has received a number of honors including: The University of Louisville Excellence in Research Award, Johnson and Johnson Fellowship for Biology, the American Society for Microbiology (ASM) Award in Applied and Environmental Microbiology, the ASM Founders Award, and the Edmund Youde Lectureship Award in Hong Kong.

He has taught a variety of courses in microbiology at the University of Louisville and has authored several textbooks in general microbiology and microbial ecology. He has written nearly 300 research papers and authored or edited more than 20 books. He has conducted studies on the fate of oil in the sea. As part of these studies, he has extensively character-

ized marine bacterial populations and examined the diversity of microorganisms. He pioneered the field of bioremediation for marine oil spills. He also has conducted studies on the application of molecular techniques to environmental problems. His studies have included the development of "suicide vectors" for the containment of genetically engineered microorganisms and the use of gene probes and the polymerase chain reaction for environmental monitoring, including the detection of pathogens and indicator bacteria for water quality monitoring.

He served as president of the American Society for Microbiology. Additionally, he has served on the National Institutes of Health (NIH) Recombinant Advisory Committee (RAC), as well as on various advisory boards for the Environmental Protection Agency (EPA), Federal Bureau of Investigation, and Department of Homeland Security. He has been chairperson of the Environmental Committee and the Task Force on Biological Weapons of the Public and Scientific Affairs Board of the American Society for Microbiology. He has been a national lecturer for Sigma Xi, an American Society for Microbiology Foundation lecturer, and an Australian Society for Microbiology national lecturer. He has served on the editorial boards of *Applied and Environmental Microbiology*, *Advances in Microbial Ecology*, *BioScience*, *Biotechniques*, *Journal of Environmental Science*, *Environmental Microbiology*, *Journal of Industrial Microbiology*, and *Biosecurity and Bioterrorism*. He is editor of *Critical Reviews in Microbiology*.



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# Introduction

## Overview

The second edition of the *Handbook of Media for Environmental Microbiology* includes descriptions of nearly 2000 media that are used for environmental microbial analyses, e.g., of water quality; for the isolation of microorganisms from soils, waters, and other environmental samples; and for the cultivation and maintenance of environmentally relevant microorganisms, including plant pathogens. It provides the formulations for the wide range of media needed to cultivate the ever expanding diversity of microorganisms that are culturable.

## Organization

The media described in the second edition of the *Handbook of Media for Environmental Microbiology* are organized alphabetically. Synonyms for media are listed. The description of each medium includes its name(s), composition, instructions for preparation, commercial sources, safety cautions where needed, and uses.

## Names of Media

Media often have numerous names. For the most part the second edition of the *Handbook of Media for Environmental Microbiology* retains the original names assigned in the literature. In some cases media with identical compositions produced by different companies have different names. For example Trypticase™ Soy Agar produced as a BBL product of BD Diagnostic Systems, Tryptone Soy Agar produced by Oxoid Unipath, and Tryptic Soy Agar produced as a Difco product of BD Diagnostic Systems have identical compositions. Many media also are known by acronyms. TSA, for example, is the common acronym for Trypticase Soy Agar.

## Trademarks

The names of some media, components of media, and other terms are registered trademarks. The trademarked items referred to in the second edition of the *Handbook of Media for Environmental Microbiology* are listed below.

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Bacto®, BiTek®, and Difco® are trademarks of Difco Laboratories (registered trademarks owned by Becton Dickinson and Company).

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## Composition of Media

Media for the cultivation of microorganisms contain the substances necessary to support the growth of microorganisms. Due to the diversity of microorganisms and their diverse metabolic pathways, there are numerous media. Even slight differences in the composition of a medium can result in dramatically different growth characteristics of microorganisms.

When methods for culturing microorganisms were first developed in the 19th century, largely by Robert Koch and his colleagues, animal and plant tissues were principally used as sources of nutrients used to support microbial growth. One of the major discoveries of Fanny Hesse in Koch's laboratory was that agar could be used to form solidified culture media on which microorganisms would grow. Extracts of plants and animal tissues were prepared as broths or mixed with agar to form a variety of culture media. Virtually any plant, animal, or animal organ was considered for use in preparing media. Infusions were prepared from beef heart, calf brains, and beef liver, as a few examples. These classic infusions still form the primary components of many media that are widely used.

The composition section of each medium describes the ingredients that make up the medium, their amounts, and the pH. It lists those ingredients in order of decreasing amount. Solids are listed first showing the weights to be added, followed by liquids showing the volumes to be included in the medium.

The composition uses generic terms where these are applicable. For example, pancreatic digest of casein is marketed by various manufacturers as trypticase, tryptone, and other commercial product names. While there may well be differences between these products, such differences are undefined. Variations also occur between batches of products produced as digests of animal tissues.

Media for the cultivation of microorganisms have a source of carbon for incorporation into biomass. For autotrophs, the carbon source most often is carbon dioxide, which may be supplied as bicarbonate within the medium. Carbohydrates, such as glucose, or other organic compounds, such as acetate, various lipids, proteins, hydrocarbons, and other organic compounds, are included in media as sources of carbon for heterotrophs. These carbon sources may also serve as the supply of energy. Other compounds, such as ammonium ions, nitrite ions, elemental sulfur, and reduced iron, may be used as the sources of energy for the cultivation of autotrophs. Nitrogen also is required for microbial growth. It may be supplied as inorganic nitrogen compounds for the cultivation of some microorganisms but more commonly is supplied as proteins, peptones, or amino acids. Phosphates and metals, such as magnesium and iron,

are also necessary components of microbiological media. Phosphates may also serve as buffers to maintain the pH of the medium within the growth tolerance limits of the microorganism being cultivated. Various additional growth factors may also be included in the media.

### Agars

Agar is the most common solidifying agent used in microbiological media. Agar is a polysaccharide extract from marine algae. It melts at 84°C and solidifies at 38°C. Agar concentrations of 15.0g/L typically are used to form solid media. Lower concentrations of 7.5–10.0g/L are used to produce soft agars or semisolid media. Below are some agars used as solidifying agents in various media.

#### Agar Bacteriological (Agar No. 1)

An agar with low calcium and magnesium. Available from Oxoid Unipath.

#### Agar, Bacto

A purified agar with reduced pigmented compounds, salts, and extraneous matter. Available from BD Diagnostic Systems.

#### Agar, BiTek™

Agar prepared as a special technical grade. Available from BD Diagnostic Systems.

#### Agar, Flake

A technical-grade agar. Available from BD Diagnostic Systems.

#### Agar, Grade A

A select-grade agar containing minerals. Available from BD Diagnostic Systems.

#### Agar, Granulated

A high-grade granulated agar that has been filtered, decolorized, and purified. Available from BD Diagnostic Systems.

#### Agar, Purified

A very high-grade agar that has been filtered, decolorized, and purified by washing and extraction of refined agars. It has reduced mineral content. Available from BD Diagnostic Systems.

#### Agar, Technical (Agar No. 3)

A technical-grade agar. Available from BD Diagnostic Systems and Oxoid Unipath.

#### Agarose

A low-sulfate neutral gelling fraction of agar that is a complex galactose polysaccharide of near neutral charge.

#### Ionagar

A purified agar. Available from Oxoid Unipath.

#### Noble Agar

An agar that has been extensively washed and is essentially free of impurities. Available from BD Diagnostic Systems.

#### Purified Agar

An agar that has been extensively washed and extracted with water and organic solvent. Available from BD Diagnostic Systems and Oxoid Unipath.

### Peptones

Many complex media, that is, media in which not all the specific chemical components are known, contain peptones as the source of nitrogen. Peptones are hydrolyzed proteins formed by enzymatic or acidic

digestion. Casein most often is used as the protein substrate for forming peptones, but other substances, such as soybean meal, also are commonly employed. Below is a list of some of the peptones that are used as ingredients in various media.

#### Acidase™ Peptone

A hydrochloric acid hydrolysate of casein. It has a nitrogen content of 8% and is deficient in cystine and tryptophan. Available from BD Diagnostic Systems.

#### Bacto Casitone

A pancreatic digest of casein. Available from BD Diagnostic Systems.

#### Bacto Peptamin

A peptic digest of animal tissues. Available from BD Diagnostic Systems.

#### Bacto Peptone

An enzymatic digest of animal tissues. It has a high concentration of low molecular weight peptones and amino acids. Available from BD Diagnostic Systems.

#### Bacto Proteose Peptone

An enzymatic digest of animal tissues. It has a high concentration of high molecular weight peptones. Available from BD Diagnostic Systems.

#### Bacto Soytone

A enzymatic hydrolysate of soybean meal. Available from BD Diagnostic Systems.

#### Bacto Triplets

An enzymatic hydrolysate containing numerous peptides, including those of higher molecular weights. Available from BD Diagnostic Systems.

#### Bacto Tryptone

A pancreatic digest of casein. Available from BD Diagnostic Systems.

#### Biosate™ Peptone

A hydrolysate of plant and animal proteins. Available from BD Diagnostic Systems.

#### Casein Hydrolysate

A hydrolysate of casein prepared with hydrochloric acid digestion under pressure and neutralized with sodium hydroxide. It contains total nitrogen of 7.6% and NaCl of 28.3%. Available from Oxoid Unipath.

#### Gelatone

A pancreatic digest of gelatin. Available from BD Diagnostic Systems.

#### Gelysate™ Peptone

A pancreatic digest of gelatin deficient in cystine and tryptophan and which has a low carbohydrate content. Available from Oxoid Unipath.

#### Lactoalbumin Hydrolysate

A pancreatic digest of lactoalbumin, a milk whey protein. It has high levels of amino acids. It contains total nitrogen of 11.9% and NaCl of 1.4%. Available from BD Diagnostic Systems and Oxoid Unipath.

#### Liver Digest Neutralized

A papaic digest of liver that contains total nitrogen of 11.0% and NaCl of 1.6%. Available from Oxoid Unipath.

#### Mycological Peptone

A peptone that contains total nitrogen of 9.5% and NaCl of 1.1%. Available from Oxoid Unipath.

**Myosate™ Peptone**

A pancreatic digest of heart muscle. Available from BD Diagnostic Systems.

**Neopeptone**

An enzymatic digest of protein. Available from BD Diagnostic Systems.

**Peptone Bacteriological Neutralized**

A mixed pancreatic and papaic digest of animal tissues. It contains total nitrogen of 14.0% and NaCl of 1.6%. Available from BD Diagnostic Systems and Oxoid Unipath.

**Peptone P**

A peptic digest of fresh meat that has a high sulfur content and contains total nitrogen of 11.12% and NaCl of 9.3%. Available from BD Diagnostic Systems and Oxoid Unipath.

**Peptonized Milk**

A pancreatic digest of high-grade skim milk powder. It has a high carbohydrate and calcium concentration. It contains total nitrogen of 5.3% and NaCl of 1.6%. Available from Oxoid Unipath.

**Phytone™ Peptone**

A papaic digest of soybean meal. It has a high vitamin and a high carbohydrate content. Available from BD Diagnostic Systems.

**Polypeptone™ Peptone**

A mixture of peptones composed of equal parts of pancreatic digest of casein and peptic digest of animal tissue. Available from BD Diagnostic Systems.

**Protease Peptone**

A specialized peptone prepared from a mixture of peptones that contains a wide variety of high molecular weight peptides. It contains total nitrogen of 12.7% and NaCl of 8.0%. Available from BD Diagnostic Systems and Oxoid Unipath.

**Protease Peptone No. 2**

An enzymatic digest of animal tissues with a high concentration of high molecular weight peptones. Available from BD Diagnostic Systems.

**Protease Peptone No. 3**

An enzymatic digest of animal tissues. It has a high concentration of high molecular weight peptones. Available from BD Diagnostic Systems.

**Soya Peptone**

A papaic digest of soybean meal with a high carbohydrate concentration. It contains total nitrogen of 8.7% and NaCl of 0.4%. Available from Oxoid Unipath.

**Soytone**

A papaic digest of soybean meal. Available from BD Diagnostic Systems.

**Special Peptone**

A mixture of peptones, including meat, plant, and yeast digests. It contains a wide variety of peptides, nucleotides, and minerals. It contains total nitrogen of 11.7% and NaCl of 3.5%. Available from Oxoid Unipath.

**Thiotone™ E Peptone**

An enzymatic digest of animal tissue. Available from BD Diagnostic Systems.

**Trypticase™ Peptone**

A pancreatic digest of casein. It has a very low carbohydrate content and a relatively high tryptophan content. Available from BD Diagnostic Systems.

**Tryptone**

A pancreatic digest of casein. It contains total nitrogen of 12.7% and NaCl of 0.4%. Available from Oxoid Unipath.

**Tryptone T**

A pancreatic digest of casein with lower levels of calcium, magnesium, and iron than tryptone. It contains total nitrogen of 11.7% and NaCl of 4.9%. Available from BD Diagnostic Systems and Oxoid Unipath.

**Tryptose**

An enzymatic hydrolysate containing high molecular weight peptides. It contains total nitrogen of 12.2% and NaCl of 5.7%. Available from BD Diagnostic Systems and Oxoid Unipath.

**Meat and Plant Extracts**

Meat and plant infusions are aqueous extracts that are commonly used as sources of nutrients for the cultivation of microorganisms. Such infusions contain amino acids and low molecular weight peptides, carbohydrates, vitamins, minerals, and trace metals. Extracts of animal tissues contain relatively high concentrations of water-soluble protein components and glycogen. Extracts of plant tissues contain relatively high concentrations of carbohydrates.

With regard to infusions, many media list as an ingredient infusion from beef heart or another animal tissue. This ingredient is prepared by boiling a given amount of the animal tissue (e.g., 500.0g), and then using the liquid or, more commonly, drying the broth and using the solids from the infusion. The actual weight of the dry solids extracted from the hot water used to create the infusion varies, and so the ingredient typically is simply listed as 500.0g beef heart infusion, although the actual weight of solids recovered from the infusion and used in the medium is far less.

Below is a list of some of the meat and plant extracts that are used as ingredients in various media.

**Bacto Beef**

A desiccated powder of lean beef. Available from BD Diagnostic Systems.

**Bacto Beef Extract**

An extract of beef (paste). Available from BD Diagnostic Systems.

**Bacto Beef Extract Desiccated**

An extract of desiccated beef. Available from BD Diagnostic Systems.

**Bacto Liver**

A desiccated powder of beef liver. Available from BD Diagnostic Systems.

**Lab-Lemco**

A meat extract powder. Available from Oxoid Unipath.

**Liver Desiccated**

Dehydrated ox livers. Available from Oxoid Unipath.

**Malt Extract**

A water-soluble extract from germinated grain dried by low-temperature evaporation. It has a high carbohydrate content. It contains total nitrogen of 1.1% and NaCl of 0.1%.



**Growth Factors**

Many microorganisms have specific growth factor requirements that must be included in media for their successful cultivation. Vitamins, amino acids, fatty acids, trace metals, and blood components often must be added to media. In some cases, specific defined components are used to meet the growth factor requirements. Incorporation of growth factors are used to enrich, that is, to increase the numbers of particular species of microorganisms. Most often, mixtures of growth factors are used in microbiological media. Acid hydrolysates of casein commonly are used as sources of amino acids. Extracts of yeast cells also are employed as sources of amino acids and vitamins for the cultivation of microorganisms. Many media, particularly those employed in the clinical laboratory, contain blood or blood components that serve as essential nutrients for fastidious microorganisms. X factor (heme) and V factor (nicotinamide adenine dinucleotide) often are supplied by adding hemoglobin, IsoVitalX, and/or Supplement VX.

**Selective Components**

Many media contain selective components that inhibit the growth of nontarget microorganisms. Selective media are especially useful in the isolation of specific microorganisms from mixed populations. In many media for the study of microorganisms in nature, compounds are included in the media as sole sources of carbon or nitrogen so that only a few types of microorganisms can grow. Selective toxic compounds are also frequently used to select for the cultivation of particular microbial species. The isolation of a pathogen from a stool specimen, for example, where there is a high abundance of non-pathogenic normal microbiota, requires selective media. Often, antimicrobics or other selectively toxic compounds are incorporated into media to suppress the growth of the background microbiota while permitting the cultivation of the target organism of interest. Bile salts, selenite, tetrathionate, tellurite, azide, phenylethanol, sodium lauryl sulfate, high sodium chloride concentrations, and various dyes—such as eosin, Crystal Violet, and Methylene Blue—are used as selective toxic chemicals. Antimicrobial agents used to suppress specific types of microorganisms include ampicillin, chloramphenicol, colistin, cycloheximide, gentamicin, kanamycin, nalidixic acid, sulfadiazine, and vancomycin. Various combinations of antimicrobics are effective in suppressing classes of microorganisms, such as enteric bacteria.

**Differential Components**

The differentiation of many microorganisms is based upon the production of acid from various carbohydrates and other carbon sources or the decarboxylation of amino acids. Some media include indicators, particularly of pH, that permit the visual detection of changes in pH resulting from such metabolic reactions.

Below is a list of some commonly used pH indicators.

pH Indicator	pH Range	Acid Color	Alkaline Color
<i>m</i> -Cresol Purple	0.5 – 2.5	Red	Yellow
Thymol Blue	1.2 – 2.8	Red	Yellow
Bromphenol Blue	3.0 – 4.6	Yellow	Blue
Bromcresol Green	3.8 – 5.4	Yellow	Blue
Chlorcresol Green	4.0 – 5.6	Yellow	Blue
Methyl Red	4.2 – 6.3	Red	Yellow
Chlorphenol Red	5.0 – 6.6	Yellow	Red
Bromcresol Purple	5.2 – 6.8	Yellow	Purple
Bromothymol Blue	6.0 – 7.6	Yellow	Blue
Phenol Red	6.8 – 8.4	Yellow	Red
Cresol Red	7.2 – 8.8	Yellow	Red
<i>m</i> -Cresol Purple	7.4 – 9.0	Yellow	Purple
Thymol Blue	8.0 – 9.6	Yellow	Blue
Cresolphthalein	8.2 – 9.8	Colorless	Red
Phenolphthalein	8.3 – 10.0	Colorless	Red

**pH Buffers**

Maintaining the pH of media usually is accomplished by the inclusion of suitable buffers. Because microorganisms grow optimally only within certain limits of a pH range, the pH generally is maintained within a few tenths of a pH unit. For the phosphate buffers, the pH is established by using varying volumes of equimolar concentrations of Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub>.

pH	Na <sub>2</sub> HPO <sub>4</sub> (mL)	NaH <sub>2</sub> PO <sub>4</sub> (mL)
5.4	3.0	97.0
5.6	5.0	95.0
5.8	7.8	92.2
6.0	12.0	88.0
6.2	18.5	81.5
6.4	26.5	73.5
6.6	37.5	62.5
6.8	50.0	50.0
7.0	61.1	38.9
7.2	71.5	28.5
7.4	80.4	19.6
7.6	86.8	13.2
7.8	91.4	8.6
8.0	94.5	5.5

**Preparation of Media**

The ingredients in a medium are usually dissolved, and the medium is then sterilized. When agar is used as a solidifying agent, the medium must be heated gently, usually to boiling, to dissolve the agar. In some cases where interactions of components, such as metals, would cause precipitates, solutions must be prepared and occasionally sterilized separately before mixing the various solutions to prepare the

complete medium. The pH often is adjusted prior to sterilization, but in some cases sterile acid or base is used to adjust the pH of the medium following sterilization. Many media are sterilized by exposure to elevated temperatures. The most common method is to autoclave the medium. Different sterilization procedures are employed when heat-labile compounds are included in the formulation of the medium.

**Tyndallization**

Exposure to steam at 100°C for 30 min will kill vegetative bacterial cells but not endospores. Such exposure can be achieved using flowing steam in an Arnold sterilizer. By allowing the medium to cool and incubate under conditions where endospore germination will occur and by repeating the 100°C–30 min exposure on 3 successive days, the medium can be sterilized because all the endospores will have germinated and the heat exposure will have killed all the vegetative cells. This process of repetitive exposure to 100°C is called tyndallization, after its discoverer, John Tyndall.

**Inspissation**

Inspissation is a heat exposure method that is employed with high-protein materials, such as egg-containing media, that cannot withstand the high temperatures used in autoclaving. This process causes coagulation of the protein without greatly altering its chemical properties. Using an Arnold sterilizer or a specialized inspissator, the medium is exposed to 75°–80°C for 2 hr on each of 3 successive days. Inspissation using an autoclave employs exposure to 85°–90°C for 10 min achieved by having a mixture of air and steam in the chamber, followed by a 15 min exposure during which the temperature is raised to 121°C using only steam under pressure in the chamber; the temperature then is slowly lowered to less than 60°C.

**Autoclaving**

Autoclaving uses exposure to steam, generally under pressure, to kill microorganisms. Exposure for 15 min to steam at 15 psi—121°C is most commonly used. Such exposure kills vegetative bacterial cells and bacterial endospores. Media containing carbohydrates often are sterilized at 116°–118°C in order to prevent the decomposition of the carbohydrate and the formation of toxic compounds that would inhibit microbial growth. Below is a list of pressure–temperature relationships.

Pressure—psi	Temperature—°C
0	100.0
1	101.9
2	103.6
3	105.3
4	106.9
5	108.4
6	109.8
7	111.3
8	112.6

9	113.9
10	115.2
11	116.4
12	117.6
13	118.8
14	119.9
15	121.0
16	122.0
17	123.0
18	124.0
19	125.0
20	126.0
21	126.9
22	127.8
23	128.7
24	129.6
25	130.4

**Filtration**

Filtration is commonly used to sterilize media containing heat-labile compounds. Liquid media are passed through sintered glass or membranes, typically made of cellulose acetate or nitrocellulose, with small pore sizes. A membrane with a pore size of 0.2mm will trap bacterial cells and, therefore, sometimes is called a bacteriological filter. By preventing the passage of microorganisms, filtration renders fluids free of bacteria and eukaryotic microorganisms, that is, free of living organisms, and hence sterile. Many carbohydrate solutions, antibiotic solutions, and vitamin solutions are filter sterilized and added to media that have been cooled to temperatures below 50°C.

**Caution about Hazardous Components**

Some media contain components that are toxic or carcinogenic. Appropriate safety precautions must be taken when using media with such components. Basic fuchsin and acid fuchsin are carcinogens, and caution must be used in handling media with these compounds to avoid dangerous exposure that could lead to the development of malignancies. Thallium salts, sodium azide, sodium biselenite, and cyanide are among the toxic components found in some media. These compounds are poisonous, and steps must be taken to avoid ingestion, inhalation, or skin contact. Azides also react with many metals, especially copper, to form explosive metal azides. The disposal of azides must avoid contact with copper or achieve sufficient dilution to avoid the formation of such hazardous explosive compounds. Media with sulfur-containing compounds may result in the formation of hydrogen sulfide, which is a toxic gas. Care must be used to ensure proper ventilation. Media with human blood or human blood components must be handled with great caution to avoid exposure to human immunodeficiency virus and other pathogens that contaminate some blood supplies.

## Uses of Media

The *Handbook of Media for Environmental Microbiology, Second Edition* contains the media used for the testing of waters and wastewaters recommended by the USEPA for the standard methods examination of water and food. It also includes the formulations of media used to cultivate the microorganisms of environmental significance that are held in the major culture collections of the world.

## References

Below is a list of references that can be consulted for further information about media used for the isolation, cultivation, and differentiation of microorganisms.

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The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. 1999. M. Dworkin, Springer-Verlag Inc. New York.

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## Web Resources

Below is a list of Web sites that provide information about media and microbial cultures.

American Type Culture Collection (ATCC), a global biological resource in the United States with many services. <http://www.atcc.org/catalogs/catalogs.html>

Belgian Coordinated Collections of Microorganisms of the Laboratorium Voor Microbiologie at the University of Gent (LMG). <http://www.belspo.be/bccm/db/media.htm>

Czechoslovakian Collection of Microorganisms. (CCM). <http://www.sci.immuni.cz/ccm>

Finish Culture Collection, Valtion Teknillinen Tutkimuskeskus (VTT). <http://www.inf.vtt.fi>

German Collection of Microorganisms (DSMZ). <http://www.gbf-braunschweig.de>

Japanese Collection of Microorganisms and Microbial Cultures. <http://www.jcm.riken.go.jp>

Netherlands Centraalbureau voor Schimmelcultures (CBS). <http://www.cbs.know.know.nl/databases>.

Russian Specialized Collection of Alkanotrophs. <http://www.ecology.psu.ru/iegmcol>

Spanish Collection of Microorganisms (Colección Española de Cultivos Tipo Catalogo de Cepas). <http://www.cect.org/english/index.htm>

United Kingdom National Collection of Yeast Cultures. <http://www.ifr.bbsrc.ac.uk/ncyc>

United Kingdom National Culture Collection Microbiological Resources. <http://www.ukncc.co.uk>

United States Food and Drug Administration Bacteriological Analytical Manual. <http://vm.cfsan.fda.gov/~embam/bam-toc.html>.

World Federation of Culture Collections. <http://www.wfcc.info>

**A 1 Broth****Composition** per liter:

Pancreatic digest of casein .....	20.0g
Lactose .....	5.0g
NaCl .....	5.0g
Salicin .....	0.5g
Triton™ X-100 .....	1.0mL

pH 6.9 ± 0.1 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into test tubes containing an inverted Durham tube. Autoclave for 10 min at 15 psi pressure–121°C.

**Use:** For the detection of fecal coliforms in treated wastewater, and seawater by a most-probable-number (MPN) method. Multiple dilutions of samples (3, 5, or 10 replicates per dilution) are added to tubes containing A 1 broth. After incubation, test tubes with gas accumulation in the Durham tubes are scored positive and those with no gas as negative. A MPN table is consulted to determine the most probable number of fecal coliforms.

**Acanthamoeba Medium****Composition** per liter:

Proteose peptone .....	15.0g
Glucose .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
L-Methionine .....	14.9mg
Thiamine .....	1.0mg
Biotin .....	0.2mg
Vitamin B <sub>12</sub> .....	1.0μg
Salt solution .....	1.0mL

pH 5.5 ± 0.2 at 25°C

**Salt Solution:****Composition** per 100.0mL:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.46g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
FeCl <sub>3</sub> .....	0.02g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.5. Filter through Whatman filter paper to remove particles. Distribute into screw-capped tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Acanthamoeba* species.

**ACC Medium****Composition** per liter:

Proteose peptone .....	20.0g
Agar .....	12.0g
Glycerol .....	1.5g
K <sub>2</sub> SO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g
Antibiotic solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C.

**Antibiotic Solution:****Composition** per 10.0mL:

Cycloheximide .....	0.075g
Ampicillin .....	0.05g
Chloramphenicol .....	0.0125g

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except antibiotic solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of fluorescent *Pseudomonas* species.

**Acetamide Agar****Composition** per liter:

Agar .....	15.0g
Acetamide .....	10.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Bromothymol Blue .....	0.08g

pH 6.9 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool tubes in a slanted position to produce a long slant.

**Use:** For the differentiation of nonfermentative Gram-negative bacteria, especially *Pseudomonas aeruginosa*. Can be used as a confirmatory test for water analysis. Bacteria that deamidate acetamide turn the medium blue.

**Acetamide Agar****Composition** per liter:

Agar .....	15.0g
Acetamide .....	10.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.39g
KH <sub>2</sub> PO <sub>4</sub> .....	0.73g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Phenol Red .....	0.012g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool tubes in a slanted position to produce a long slant.

**Use:** For the differentiation of nonfermentative Gram-negative bacteria, especially *Pseudomonas aeruginosa*. Can be used as a confirmatory test for water analysis. Bacteria that deamidate acetamide turn the medium blue.

**Acetamide Broth****Composition** per liter:

Acetamide .....	10.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.39g
KH <sub>2</sub> PO <sub>4</sub> .....	0.73g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Phenol Red .....	0.012g
pH 6.9 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the differentiation of nonfermentative Gram-negative bacteria, especially *Pseudomonas aeruginosa*. Can be used as a confirmatory test for water analysis. Bacteria that deaminate acetamide turn the broth purplish red.

#### Acetate Agar

**Composition** per liter:

Agar .....	15.0g
Yeast extract .....	2.0g
Sodium acetate .....	1.0g
Pancreatic digest of casein .....	1.0g
pH 7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Caryophanon latum*.

#### Acetate Differential Agar

(Sodium Acetate Agar)

(Simmons' Citrate Agar, Modified)

**Composition** per liter:

Agar .....	20.0g
NaCl .....	5.0g
Sodium acetate .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Bromothymol Blue .....	0.08g
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to cold distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes to produce a 1 cm butt and 30 cm slant. Autoclave for 15 min at 15 psi pressure–121°C. Cool tubes in a slanted position.

**Use:** For the differentiation of *Shigella* species from *Escherichia coli* and also for the differentiation of nonfermenting Gram-negative bacteria. Bacteria that can utilize acetate as the sole carbon source turn the medium blue.

#### Acetivibrio cellulolyticus Medium

**Composition** per 1170.0mL:

Cellobiose or cellulose (MN 300, Whatman CF II, Kleenex tissue paper, or HCl-treated cotton) .....	3.0g
NaHCO <sub>3</sub> .....	2.0g
L-Cysteine-HCl .....	0.25g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
Resazurin .....	0.001g
Mineral solution 1 .....	75.0mL
Mineral solution 2 .....	75.0mL
Cellobiose solution .....	50.0mL
Trace elements solution .....	10.0mL

Vitamin solution .....	10.0mL
Reducing agent solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

#### Mineral Solution 1:

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	3.9g
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**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Mineral Solution 2:

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.72g
NaCl .....	0.59g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Cellobiose Solution:

**Composition** per 50.0mL:

D-Cellobiose .....	5.0g
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**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

#### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add distilled/deionized water to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 7.0 with 1N KOH.

#### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Reducing Agent Solution:

## Composition per 110.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	2.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.5g

**Preparation of Reducing Agent Solution:** Add 110.0mL of distilled/deionized water to a 250.0mL flask. Boil under N<sub>2</sub> gas for 1 min. Cool to room temperature. Add L-cysteine-HCl·H<sub>2</sub>O and dissolve. Adjust to pH 9 with 5N NaOH. Add washed Na<sub>2</sub>S·9H<sub>2</sub>O and dissolve. Distribute under N<sub>2</sub> gas in 10.0mL volumes into tubes. Autoclave for 10 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except cellobiose solution and reducing agent solution, to distilled/deionized water and bring volume to 940.0mL. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 7.6 by gassing. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. After autoclaving, the pH of the medium will be 7.2. Prior to inoculation of cultures, aseptically and anaerobically add 0.1mL of sterile reducing agent solution and 0.5mL of sterile cellobiose solution to each tube containing 9.4mL of sterile basal medium.

**Use:** For the cultivation and maintenance of *Acetivibrio cellulolyticus*, an anaerobic, cellulolytic bacterium.

## *Acetivibrio Desulfovibrio* Medium (LMG Medium 105)

### Composition per liter:

Solution A .....	869.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E .....	10.0mL
Solution F .....	10.0mL
Solution B .....	1.0mL

pH 7.7 ± 0.2 at 25°C

### Solution A:

#### Composition per 869.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 869.0mL. Mix thoroughly. Prepare and autoclave part A under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Solution B:

#### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
HCl, 25% .....	10.0mL

**Preparation of Solution B:** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to the HCl. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Auto-

clave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Solution C:

#### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove residual O<sub>2</sub>.

### Solution D:

#### Composition per 10.0mL:

Sodium butyrate .....	0.7g
Sodium caproate .....	0.3g
Sodium octanoate .....	0.15g

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Solution E:

#### Composition per 10.0mL:

Yeast extract .....	1.0g
Thiamine-HCl .....	100.0µg
p-Aminobenzoic acid .....	40.0µg
D(+)-Biotin .....	10.0µg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Solution F:

#### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** To 869.0mL of sterile cooled Part A, aseptically add the remaining sterile solutions in the following order: solution B, solution C, solution D, solution E, and solution F. Mix thoroughly. Adjust pH to 7.7. Anaerobically distribute under 90% N<sub>2</sub> + 10% CO<sub>2</sub> into sterile tubes or flasks.

**Use:** For the cultivation of *Acetivibrio ethanologignens* and *Desulfovibrio sapovorans* which reduces sulfate.

## *Acetobacter diazotrophicus* Agar

### Composition per liter:

Glucose .....	50.0g
CaCO <sub>3</sub> .....	30.0g
Agar .....	25.0g
Yeast extract .....	10.0g

pH 5.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly to evenly distribute CaCO<sub>3</sub>. Bring pH to 5.5. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool rapidly to 50°–55°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Acetobacter diazotrophicus*, a nitrogen fixing endophyte.

***Acetobacterium dehalogenans* Medium  
(DSMZ Medium 787)****Composition per liter:**

NaHCO <sub>3</sub> .....	10.0g
Yeast extract.....	2.0g
NH <sub>4</sub> Cl.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.45g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Resazurin.....	1.0mg
NaHCO <sub>3</sub> solution.....	30.0mL
Na <sub>2</sub> CO <sub>3</sub> solution.....	20.0mL
Trace elements solution.....	20.0mL
Vitamin solution.....	20.0mL
Na-syringate solution.....	10.0mL
Cysteine solution.....	10.0mL

pH 7.4 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Na-syringate Solution:****Composition per 20.0mL:**

Na-syringate.....	1.2g
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**Preparation of Na-syringate Solution:** Add Na-syringate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitritotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl.....	10.0mg
Thiamine·HCl·2H <sub>2</sub> O.....	5.0mg

Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>CO<sub>3</sub> solution, vitamin solution, Na-syringate solution, and cysteine solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute 9.0mL aliquots into serum bottles. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add approximately 0.20mL sterile Na<sub>2</sub>CO<sub>3</sub> solution to each 9.0mL of medium so that pH is adjusted to 7.4. For every 9.0mL of medium inject 1.0mL NaHCO<sub>3</sub> solution, 0.15mL Na-syringate solution, 0.2mL vitamin solution, and 0.17mL cysteine solution.

**Use:** For the cultivation of *Acetobacterium dehalogenans*, which dehalogenates methyl chloride.

***Acetobacterium tundrae* Medium  
(DSMZ Medium 900)****Composition per liter:**

Yeast extract.....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.52g
KCl.....	0.33g
NH <sub>4</sub> Cl.....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.3g
Resazurin.....	0.5mg
Fructose solution.....	30.0mL
NaHCO <sub>3</sub> solution.....	20.0mL
L-Cysteine solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace mineral solution SL-10.....	1.0mL
Seven vitamin solution.....	1.0mL

pH 7.0 ± 0.2 at 25°C

**L-Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
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**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Fructose Solution:

**Composition** per 30.0mL:

Glucose ..... 5.0g

**Preparation of Fructose Solution:** Add glucose to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Seven Vitamin Solution:

**Composition** per liter:

Pyridoxine hydrochloride ..... 300.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 200.0mg  
Nicotinic acid ..... 200.0mg  
Vitamin B<sub>12</sub> ..... 100.0mg  
Calcium pantothenate ..... 100.0mg  
*p*-Aminobenzoic acid ..... 80.0mg  
D(+)-Biotin ..... 20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except seven vitamin solution, NaHCO<sub>3</sub> solution, fructose solution, L-Cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 929.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium, 1.0mL seven vitamin solution, 20.0mL NaHCO<sub>3</sub> 30.0mL fructose solution, 10.0mL L-Cysteine-HCl·H<sub>2</sub>O solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Acetobacterium tundrae*, a psychrophilic, acetogenic bacterium from tundra soils.

### Acetogen Medium

**Composition** per 421.8mL:

NaHCO<sub>3</sub> ..... 2.4g  
NH<sub>4</sub>Cl ..... 0.2g  
Yeast extract ..... 0.2g  
Stock salts solution #1 ..... 40.0mL  
Potassium phosphate buffer ..... 20.0mL  
Clarified rumen fluid ..... 20.0mL  
Stock salts solution #2 ..... 4.0mL  
Trace minerals ..... 4.0mL

Vitamin solution ..... 4.0mL  
Reducing agent ..... 4.0mL  
Tungstate solution ..... 0.4mL  
Resazurin (0.1% solution) ..... 0.4mL

#### Potassium Phosphate Buffer:

**Composition** per 830.0mL:

K<sub>2</sub>HPO<sub>4</sub> ..... 15.68g  
KH<sub>2</sub>PO<sub>4</sub> ..... 4.72g

**Preparation of Potassium Phosphate Buffer:** Dissolve K<sub>2</sub>HPO<sub>4</sub> in 600.0mL of distilled/deionized water and KH<sub>2</sub>PO<sub>4</sub> in 230.0mL of distilled/deionized water. Mix the two solutions together and use.

#### Stock Salts Solution #1:

**Composition** per liter:

KCl ..... 1.6g  
NaCl ..... 1.4g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

**Preparation of Stock Salts Solution #1:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Stock Salts Solution #2:

**Composition** per liter:

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g

**Preparation of Stock Salts Solution #2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Minerals:

**Composition** per liter:

Nitrilotriacetic acid ..... 1.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.5g  
NaCl ..... 1.0g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub> ..... 0.1g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.01g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
AlK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.01g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Minerals:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Bring volume to 1.0L with distilled/deionized water. Add remaining components. Mix thoroughly.

#### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Ascorbic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
Choline chloride ..... 5.0mg  
Lipoic acid ..... 5.0mg  
*i*-Inositol ..... 5.0mg  
Niacinamide ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Pyridoxal-HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.1mg



**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Store frozen.

**Tungstate Solution:**

**Composition** per liter:

Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O ..... 99.0mg

**Preparation of Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Reducing Agent:**

**Composition** per 110.0mL:

L-Cysteine-HCl·H<sub>2</sub>O ..... 2.5g

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 2.5g

**Preparation of Reducing Agent:** Add 110.0mL of distilled/deionized water to a 250.0mL round-bottomed flask. Boil under N<sub>2</sub> gas for 1 min. Cool to room temperature. Add L-cysteine-HCl and dissolve. Adjust to pH 9 with 5N NaOH. Add washed Na<sub>2</sub>S·9H<sub>2</sub>O and dissolve. Distribute in amounts needed into tubes or flasks. Autoclave for 10 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> and reducing agent, to distilled/deionized water and bring volume to 417.8mL. Mix thoroughly. Gently heat and bring to boiling under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Cool to 45°–50°C. Add NaHCO<sub>3</sub> and reducing agent. Distribute into tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. After inoculation, exchange headspace with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of acetogenic anaerobes such as some *Clostridium* species.

**Acetohalobium Medium**

**Composition** per 1025.0mL:

NaCl ..... 150.0g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 4.0g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.33g

KCl ..... 0.33g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.33g

NH<sub>4</sub>Cl ..... 0.33g

Resazurin ..... 1.0mg

Trace elements solution ..... 10.0mL

Vitamin solution ..... 10.0mL

Trimethylamine-HCl solution ..... 10.0mL

Yeast extract solution ..... 5.0mL

NaHCO<sub>3</sub> solution ..... 5.0mL

Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 5.0mL

pH 7.6 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g

Nitrilotriacetic acid ..... 1.5 g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.0g

NaCl ..... 1.0g

MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g

CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g

KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g

CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g

H<sub>3</sub>BO<sub>3</sub> ..... 0.01g

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add distilled/deionized water to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 7.0 with KOH.

**Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg

Calcium DL-pantothenate ..... 5.0mg

Lipoic acid ..... 5.0mg

Nicotinic acid ..... 5.0mg

p-Aminobenzoic acid ..... 5.0mg

Riboflavin ..... 5.0mg

Thiamine-HCl ..... 5.0mg

Biotin ..... 2.0mg

Folic acid ..... 2.0mg

Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Trimethylamine-HCl Solution:**

**Composition** per 10.0mL:

Trimethylamine-HCl ..... 2.4g

**Preparation of Trimethylamine-HCl Solution:** Add trimethylamine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas. One may use 4.5g of glycine betaine in place of trimethylamine-HCl.

**Yeast Extract Solution:**

**Composition** per 5.0mL:

Yeast extract ..... 0.05g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 5.0mL:

NaHCO<sub>3</sub> ..... 0.5g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 5.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O solution to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

**Preparation of Medium:** Add components, except vitamin solution, trimethylamine-HCl solution, yeast extract solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.2–7.4 with NaOH. Mix thoroughly. Gently heat and bring to boiling. Boil for a few minutes. Allow to cool to room temperature under 100% N<sub>2</sub>. Distribute into tubes or flasks under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Before inoculation, aseptically and anaerobically add vitamin solution, trimethylamine-HCl solution, yeast extract solution, NaHCO<sub>3</sub> solu-

tion, and  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution. If necessary, adjust pH 7.6 to with sterile anaerobic  $\text{Na}_2\text{CO}_3$  solution.

**Use:** For the cultivation and maintenance of *Acetohalobium arabaticum*, which is capable of chemolithotrophic growth on  $\text{H}_2 + \text{CO}_2$  or  $\text{CO}$ , of methylotrophic growth on trimethylamine, and of organotrophic growth on betaine, lactate, etc.

#### Acetylglucosamine Medium (N-Acetylglucosamine Medium)

**Composition per liter:**

N-Acetylglucosamine.....	20.0g
Beef extract.....	10.0g
Peptone.....	10.0g
Yeast extract.....	5.0g
$\text{K}_2\text{HPO}_4$ .....	2.0g
Triammonium citrate.....	2.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.2g
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ .....	0.05g
Tween™ 80.....	1.0mL

pH  $6.2 \pm 0.4$  at  $25^\circ\text{C}$

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure– $121^\circ\text{C}$ .

**Use:** For the cultivation of bacteria that can utilize N-acetylglucosamine.

#### Acid Glucose Salts Medium

**Composition per liter:**

Glucose.....	5.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5g
$(\text{NH}_4)_2\text{SO}_4$ .....	0.15g
$\text{KH}_2\text{PO}_4$ .....	0.1g
KCl.....	50.0mg
$\text{Ca}(\text{NO}_3)_2$ .....	10.0mg

pH  $3.0 \pm 0.2$  at  $25^\circ\text{C}$

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure– $121^\circ\text{C}$ .

**Use:** For the cultivation of *Thiobacillus organoparus*.

#### Acid Rhodospirillaceae Medium

**Composition per 1050.0 mL:**

Ammonium acetate.....	1.5g
$\text{KH}_2\text{PO}_4$ .....	0.5g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.4g
NaCl.....	0.4g
$\text{NH}_4\text{Cl}$ .....	0.4g
Disodium succinate.....	0.25g
Yeast extract.....	0.2g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.05g
Ferric citrate solution.....	5.0mL
Trace elements solution SL-6.....	1.0mL
Vitamin $\text{B}_{12}$ solution.....	0.4mL
Neutralized sulfide solution.....	variable

pH  $5.7 \pm 0.2$  at  $25^\circ\text{C}$

**Ferric Citrate Solution:**

**Composition per 10.0mL:**

Ferric citrate.....	10.0mg
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3

min. Autoclave for 15 min at 15 psi pressure– $121^\circ\text{C}$ . Store under  $\text{N}_2$  gas.

#### Trace Elements Solution SL-6:

**Composition per liter:**

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	0.5g
$\text{H}_3\text{BO}_3$ .....	0.3g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.2g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	0.03g
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.02g
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin $\text{B}_{12}$ Solution:

**Composition per 100.0mL:**

Vitamin $\text{B}_{12}$ .....	10.0mg
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**Preparation of Vitamin  $\text{B}_{12}$  Solution:** Add Vitamin  $\text{B}_{12}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure– $121^\circ\text{C}$ . Store under  $\text{N}_2$  gas.

#### Neutralized Sulfide Solution:

**Composition per 100.0mL:**

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ .....	1.5g
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**Preparation of Neutralized Sulfide Solution:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure– $121^\circ\text{C}$ . Cool to room temperature. Adjust pH to about 7.3 with sterile 2M  $\text{H}_2\text{SO}_4$ . Do not open the bottle to add  $\text{H}_2\text{SO}_4$ ; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add components, except neutralized sulfide solution, to distilled/deionized water and bring volume to 1050.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3–4 min under a stream of 100%  $\text{N}_2$ . Distribute 45.0mL of the prepared medium into 50.0mL screw-capped tubes that have been flushed with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure– $121^\circ\text{C}$ . Cool to room temperature. Before inoculation, aseptically and anaerobically add 0.25–0.50mL of neutralized sulfide solution.

**Use:** For the cultivation and maintenance of members of the family Rhodospirillaceae, including *Rhodomicrobium vannielii* and *Rhodopseudomonas acidophila*.

#### Acid Tomato Broth

**Composition per liter:**

Glucose.....	10.0g
Peptone.....	10.0g
Yeast extract.....	5.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.2g
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ .....	0.05g
Tomato juice.....	250.0mL
L-Cysteine solution.....	0.5mL

**L-Cysteine Solution:**

**Composition per 10.0mL:**

L-Cysteine.....	0.1g
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**Preparation of L-Cysteine Solution:** Add 0.1g of L-cysteine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine solution, to distilled/deionized water and bring volume to 999.5mL. Mix thoroughly. Adjust pH to 4.8. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.5mL of sterile L-cysteine solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of a variety of fungi.

#### *Acidaminobacter Medium*

**Composition per liter:**

NaHCO <sub>3</sub> .....	2.0g
Glycine.....	1.5g
NaCl.....	1.2g
KCl.....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Na <sub>2</sub> SO <sub>4</sub> .....	0.2g
Yeast extract.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	30.0μg
Vitamin solution.....	10.0mL
NaHCO <sub>3</sub> solution.....	5.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	5.0mL
Trace elements solution SL-10.....	1.0mL

pH 7.4 ± 0.2 at 25°C

#### **Trace Elements Solution SL-10:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

#### **Vitamin Solution:**

**Composition per liter:**

Pyridoxine·HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine·HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### **NaHCO<sub>3</sub> Solution:**

**Composition per 5.0mL:**

NaHCO <sub>3</sub> .....	0.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 5.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O solution to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

**Preparation of Medium:** Add components, except vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Boil for a few minutes. Allow to cool to room temperature under 100% N<sub>2</sub>. Distribute into tubes or flasks under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Before inoculation, aseptically and anaerobically add vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Check that final pH is 7.4.

**Use:** For the cultivation and maintenance of *Acidaminobacter hydrogeniformans*, a fermentative bacterium from estuarine mud.

#### *Acidaminococcus fermentans Medium*

**Composition per liter:**

Casamino acids.....	10.0g
Glucose.....	5.0g
Pancreatic digest of casein.....	5.0g
Yeast extract.....	5.0g
Sodium glutamate.....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
Arginine.....	1.0g
Glycine.....	1.0g
L-Cysteine·HCl.....	0.5g
DL-Tryptophan.....	0.1g
Tween™ 80.....	0.5mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Acidaminococcus fermentans* from rumen.

#### *Acidaminococcus Medium VR*

**Composition per liter:**

Acid-hydrolyzed casein (vitamin and salt free).....	20.0g
Glucose.....	5.0g
L-Cysteine·HCl·H <sub>2</sub> O.....	0.35g
DL-Tryptophan.....	0.1g
Guanine.....	0.01g
Uracil.....	0.01g
Hypoxanthine.....	0.01g
Pyridoxal.....	1.0mg
Calcium pantothenate.....	1.0mg
Thiamine.....	50.0μg
Niacin.....	50.0μg
Riboflavin.....	50.0μg
<i>p</i> -Aminobenzoic acid.....	10.0μg
Biotin.....	2.0μg

Folic acid.....	1.0μg
Vitamin B <sub>12</sub> .....	1.0μg
VR salts A.....	30.0mL
VR salts B.....	4.0mL
pH 7.0 ± 0.2 at 25°C	

**VR Salts A:****Composition per 500.0mL:**

Na <sub>2</sub> HPO <sub>4</sub> .....	37.5g
KH <sub>2</sub> PO <sub>4</sub> .....	12.5g

**Preparation of VR Salts A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**VR Salts B:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	24.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.25g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.25g
VSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.25g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.125g

**Preparation of VR Salts B:** Add components to distilled/deionized water and bring volume to 700.0mL. Add 2.0mL of concentrated HCl and heat until dissolved. Add 5.0g of nitrilotriacetic acid to 300.0mL distilled/deionized water. Adjust pH with 10N NaOH to 7.0. Stir vigorously. Slowly add the nitrilotriacetic acid solution to the larger volume of salt solutions while stirring until dissolved. Add distilled/deionized water and bring volume to 1.0L. Filter through paper. Store in a cool place.

**Preparation of Medium:** Filter sterilize vitamins as separate solution. Add aseptically to sterile basal medium. If necessary, adjust pH with solid K<sub>2</sub>CO<sub>3</sub> to 7.0. Prepare and distribute medium anaerobically using Hungate techniques with 100% N<sub>2</sub> gas.

**Use:** For the cultivation and maintenance of *Acidaminococcus fermentans* from rumen.

***Acidianus brierleyi* Medium****Composition per liter:**

Sulfur flowers.....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
KCl.....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g
Yeast extract solution.....	10.0mL

pH 1.5–2.5 at 25°C

**Yeast Extract Solution:****Composition per 10.0mL:**

Yeast extract.....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except sulfur flowers and yeast extract solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Sulfur flowers are sterilized separately by steaming for 3 hr on 3 consecutive days. Aseptically com-

bine the basal solution, sterile sulfur flowers, and sterile yeast extract solution. Adjust pH to 1.5–2.5 with 6N H<sub>2</sub>SO<sub>4</sub>.

**Use:** For the cultivation and maintenance of *Acidianus brierleyi*, a facultatively anaerobic thermoacidophilic archaean.

***Acidianus infernus* Medium****Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Yeast extract.....	1.0g
Sulfur flowers.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

pH 2.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2.5 with 10N H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the aerobic cultivation and maintenance of *Acidianus infernus*, *Acidianus brierleyi*, *Acidianus infernus*, and *Desulfurolobus ambivalens*.

**Acidic Rhodospirillaceae Medium****Composition per 1006.0mL:**

Disodium succinate.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4g
NaCl.....	0.4g
NH <sub>4</sub> Cl.....	0.4g
Yeast extract.....	0.2g
CaCl <sub>2</sub> ·H <sub>2</sub> O.....	50.0mg
Ferric citrate solution.....	5.0mL
Trace elements solution.....	1.0mL

**Ferric Citrate Solution:****Composition per 100.0mL:**

Ferric citrate.....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ferric citrate solution and trace elements solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically

add 5.0mL of sterile ferric citrate solution and 1.0mL of sterile trace elements solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Rhodospseudomonas acidophila*.

#### **Acidic Tomato Medium for *Leuconostoc***

**Composition per liter:**

Agar .....	15.0g
Glucose .....	10.0g
Peptone.....	10.0g
Yeast extract.....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.05g
Tomato juice.....	250.0mL

pH 4.8 ± 0.2 at 25°C

**Preparation of Medium:** Add solid components to 750.0mL of distilled/deionized water. Add tomato juice. Mix well and warm gently until dissolved. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and maintenance of *Leuconostoc oenos* and other *Leuconostoc* species.

#### **Acidimicrobium Medium**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.4g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
KCl.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
Yeast extract solution .....	20.0mL

pH 2.0 ± 0.2 at 25°C

**Yeast Extract Solution:**

**Composition per 20.0mL:**

Yeast extract.....	10.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except yeast extract solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 2.0 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile yeast extract solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the heterotrophic cultivation of *Sulfobacillus acidophilus*.

#### **Acidimicrobium Medium**

**Composition per liter:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	13.9g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.4g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
KCl.....	0.1g

pH 1.7 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 1.7 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the autotrophic cultivation of *Sulfobacillus acidophilus*.

#### **Acidiphilium Medium**

**Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl.....	0.1g
Glucose solution.....	10.0mL
Yeast extract solution .....	10.0mL

pH 3.0 ± 0.2 at 25°C

**Glucose Solution:**

**Composition per 10.0mL:**

D-Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Yeast Extract Solution:**

**Composition per 10.0mL:**

Yeast extract .....	0.3g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution and yeast extract solution, to distilled/deionized water and bring volume to 1080.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 3.0 using 1N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Before inoculation, aseptically add glucose solution and yeast extract solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Acidiphilium cryptum*.

#### **Acidobacterium Medium**

**Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Glucose.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl.....	0.1g
Yeast extract .....	0.1g

pH 3.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.5 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Acidobacterium capsulatum*.

#### **Acidolobus aceticus Medium (DSMZ Medium 901)**

**Composition per 1055mL:**

Sulfur, powdered .....	10.0g
NH <sub>4</sub> Cl.....	0.33g
KCl.....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
Resazurin .....	0.5mg
Yeast extract solution .....	30.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	15.0mL
Vitamin solution .....	10.0mL
Trace mineral solution SL-10 .....	1.0mL

pH 3.5–3.8 at 25°C

# Trace Elements Solution SL-10:

## Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

## Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

## Vitamin Solution:

### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

## Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

### Composition per 20.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature. Neutralize to pH 7.0 with HCl.

## Yeast Extract Solution:

### Composition per 30.0mL:

Yeast extract .....	3.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under 100% CO<sub>2</sub>. Add components, except vitamin solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and yeast extract solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.5 with H<sub>2</sub>SO<sub>4</sub>. Distribute to anaerobe tubes or bottles. Heat to 90°C for 1 h on each of 3 successive days. Aseptically and anaerobically add per liter of medium, 10.0mL sterile vitamin solution, 15.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 30.0mL sterile yeast extract solution. Mix thoroughly. The final pH should be 3.5–3.8.

**Use:** For the cultivation of *Acidilobus aceticus*, a novel anaerobic thermoacidophilic archaeon from continental hot vents in Kamchatka.

# *Acidomonas* Agar

## Composition per liter:

Solution A .....	500.0mL
Solution B .....	500.0mL

## Solution A:

### Composition per 500.0mL:

Glucose .....	10.0g
Peptone .....	5.0g
Malt extract .....	3.0g
Yeast extract .....	3.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

## Solution B:

### Composition per 500.0mL:

Agar .....	20.0g
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**Preparation of Solution B:** Add 20.0g of agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically mix 500.0mL of solution A with 500.0mL of solution B. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Acidomonas methanolica*, an acidophilic facultatively methylotrophic bacterium.

# *Acidomonas methanolica* Agar

## Composition per liter:

Solution A .....	500.0mL
Solution B .....	500.0mL

pH 4.0 ± 0.2 at 25°C

## Solution A:

### Composition per 500.0mL:

Glucose .....	20.0g
Yeast extract .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.7g
NaCl .....	0.5g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.4g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.16g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

## Solution B:

### Composition per 500.0mL:

Agar .....	20.0g
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**Preparation of Solution B:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically mix 500.0mL of solution A and 500.0mL of solution B. Mix thoroughly. Aseptically adjust pH to 4.0. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Acidomonas methanolica*, an acidophilic facultatively methylotrophic bacterium.

**Acidophilic *Bacillus stearothermophilus* Agar****Composition per liter:**

Part B .....	600.0mL
Part A .....	400.0mL

pH 5.0 ± 0.2 at 25°C

**Part A:****Composition per 400.0mL:**

Soluble starch .....	10.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g

**Preparation of Part A:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 4.7. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Part B:****Composition per 600.0mL:**

Agar .....	20.0g
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**Preparation of Part B:** Add agar to distilled/deionized water and bring volume to 600.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Preparation of Medium:** Aseptically combine solution A and solution B. Mix thoroughly. Adjust pH to 5.0. Pour into sterile Petri dishes.

**Use:** For the cultivation and maintenance of *Bacillus stearothermophilus* and other acidophilic *Bacillus* species.

**Acidophilic *Bacillus stearothermophilus* Broth****Composition per liter:**

Soluble starch .....	10.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g

pH 5.0 ± 0.2 at 25°C

**Preparation of Medium:** Dissolve all components in 1.0L of distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Adjust to pH 5.0. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Bacillus stearothermophilus* and other acidophilic *Bacillus* species.

**Acidophilium Agar****Composition per liter:**

Solution A .....	500.0mL
Solution B .....	500.0mL

pH 3.5 ± 0.2 at 25°C

**Solution A:****Composition per 500.0mL:**

Agar .....	12.0g
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**Preparation of Solution A:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Solution B:****Composition per 500.0mL:**

Mannitol .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
Tryptone soya broth .....	0.1g
KCl .....	50.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	50.0mg
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 3.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Preparation of Medium:** Aseptically combine 500.0mL of solution A with 500.0mL of solution B. Mix thoroughly. Pour into sterile Petri dishes or aseptically distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Acidiphilium cryptum* and other *Acidiphilium* species.

**Activated Carbon Medium (DSMZ Medium 811)****Composition per liter:**

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
Activated carbon .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	20.0mg
NH <sub>4</sub> -Fe-III-Citrate .....	1.2mg
Trace elements solution TS2 .....	1.0mL

pH 7.5 ± 0.1 at 25°C

**Trace Elements Solution TS2:****Composition per liter:**

Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	900.0mg
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	200.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	100.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except activated carbon, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. After all components are dissolved add activated carbon. Bring volume to 1.0L with distilled/deionized water. Adjust pH to 7.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into Petri dishes or leave in tubes.

**Use:** For the cultivation of *Streptomyces thermoautotrophicus*.

**Aeromonas Differential Agar (Dextrin Fuchsin Sulfite Agar)****Composition per liter:**

Dextrin .....	15.0g
Agar .....	13.0g
Pancreatic digest of casein .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	7.75g
NaCl .....	5.0g
Beef extract .....	3.0g
Na <sub>2</sub> SO <sub>3</sub> .....	1.6g
Acid Fuchsin solution .....	50.0mL

pH 7.5 ± 0.2 at 25°C

# **Acid Fuchsin Solution:**

## **Composition per 50.0mL:**

Acid Fuchsin .....	0.25g
Aquesou dioxan, 5% .....	50.0mL

**Preparation of Acid Fuchsin Solution:** Add Acid Fuchsin to 50.0mL of 5% aqueous dioxan. Mix well to dissolve.

**Caution:** Acid Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contamination of the skin.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and differentiation of *Aeromonas* species from other Gram-negative rods such as *Pseudomonas* and Enterobacteriaceae. Specimens with low numbers of *Aeromonas* may first be enriched by growth in starch broth for 4–9 days. After 24 hrs of growth on this agar, colonies are sprayed with Nadi reagent (1% solution of *N,N,N',N'*-tetramethyl-*p*-phenylene-diammonium dichloride). A positive Nadi reaction (dextrin degradation) is indicated by a purple color at the periphery of the colony. Dextrin fermentation is also indicated by red colonies. *Aeromonas* species appear as large, convex, dark red colonies with a purple periphery.

## ***Aeromonas hydrophila* Medium**

### **Composition per liter:**

Inositol .....	10.0g
Pancreatic digest of casein .....	10.0g
L-Ornithine-HCl .....	5.0g
Proteose peptone .....	5.0g
Agar .....	3.0g
Yeast extract .....	3.0g
Mannitol .....	1.0g
Ferric ammonium citrate .....	0.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.4g
Bromocresol Purple .....	0.02g

pH 6.7 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 6.7. Distribute into tubes in 5.0mL volumes. Autoclave for 12 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Aeromonas hydrophila*.

## ***Aeromonas* Medium (Ryan's *Aeromonas* Medium)**

### **Composition per liter:**

Agar .....	12.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	10.67g
Proteose peptone .....	5.0g
NaCl .....	5.0g
Xylose .....	3.75g
L-Lysine-HCl .....	3.5g
Yeast extract .....	3.0g
Sorbitol .....	3.0g
Bile salts No. 3 .....	3.0g
Inositol .....	2.5g
L-Arginine-HCl .....	2.0g
Lactose .....	1.5g
Ferric ammonium citrate .....	0.8g

Bromothymol Blue .....	0.04g
Thymol Blue .....	0.04g
pH 8.0 ± 0.1 at 25°C	

**Source:** This medium is available as a dehydrated powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 50°C and aseptically add 5.0mg of ampicillin. Pour into sterile Petri dishes.

**Use:** For the isolation and selective differentiation of *Aeromonas hydrophila* and other *Aeromonas* species. *Aeromonas* species appear as small (0.5–1.5mm), dark green colonies with darker centers.

## ***Agrobacterium* Agar**

### **Composition per liter:**

Agar .....	15.0g
Mannitol .....	8.0g
NaCl .....	5.0g
Yeast extract .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Casamino acids .....	0.5g

pH 6.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.6. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens*.

## ***Agrobacterium* Agar**

### **Composition per liter:**

Agar .....	15.0g
Glucose .....	10.0g
Yeast extract .....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.25g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Agrobacterium azotophilum*, *Agrobacterium radiobacter*, *Agrobacterium rhizogenes*, *Agrobacterium rubi*, *Agrobacterium tumefaciens*, and *Agrobacterium vitis*.

## ***Agrobacterium* Agar with Biotin**

### **Composition per liter:**

Agar .....	15.0g
Mannitol .....	8.0g
NaCl .....	5.0g
Yeast extract .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Casamino acids .....	0.5g
Biotin solution .....	0.1mL

pH 6.6 ± 0.2 at 25°C

### **Biotin Solution:**

#### **Composition per 10.0mL:**

Biotin .....	0.2mg
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**Preparation of Biotin Solution:** Add biotin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except biotin solution, to distilled/deionized water and bring volume to 999.9mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 0.1mL of sterile biotin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of slow-growing *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens*.

#### *Agrobacterium Mannitol Medium*

**Composition per liter:**

Mannitol .....	10.0g
L-Glutamate .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Yeast extract .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Agrobacterium rhizogenes*.

#### *Agrobacterium Medium*

**Composition per liter:**

Agar .....	20.0g
Mannitol .....	10.0g
NaNO <sub>3</sub> .....	4.0g
MgCl <sub>2</sub> .....	2.0g
Calcium propionate .....	1.2g
Mg <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	0.2g
MgSO <sub>4</sub> .....	0.1g
MgCO <sub>3</sub> .....	0.075g
NaHCO <sub>3</sub> .....	0.075g
Supplement .....	100.0mL

pH 7.1 ± 0.2 at 25°C

#### **Supplement:**

**Composition per 100.0mL:**

Berberine .....	0.275g
Cycloheximide .....	0.2g
Bacitracin .....	0.1g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.1g
Penicillin G .....	0.06g
Streptomycin sulfate .....	0.03g
Tyrosine .....	1.0mg

**Preparation of Supplement:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except supplement, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile supplement. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of *Agrobacterium* species.

#### *Agrobacterium Medium D1*

**Composition per liter:**

Agar .....	15.0g
Mannitol .....	15.0g
LiCl .....	6.0g
NaNO <sub>3</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Bromothymol Blue .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.02g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Adjust pH to 7.2. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation and cultivation of *Agrobacterium* species.

#### *Agrobacterium tumefaciens*

#### **Modified Roy and Sasser Medium for Grapevine Strains**

**Composition per 1020mL:**

Agar .....	15.0g
Adonitol .....	4.0g
H <sub>3</sub> BO <sub>3</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.7g
NaCl .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Yeast extract .....	0.14g
Cycloheximide solution .....	10.0mL
Triphenyl tetrazolium chloride solution .....	1.0mL
D-Cycloserine solution .....	1.0mL
Trimethoprim solution .....	1.0mL

#### **Cycloheximide Solution:**

**Composition per 10.0mL:**

Cycloheximide .....	0.025g
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**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Triphenyl Tetrazolium Chloride Solution:**

**Composition per 10.0mL:**

Triphenyl tetrazolium chloride .....	0.8g
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**Preparation of Triphenyl Tetrazolium Chloride Solution:** Add triphenyl tetrazolium chloride to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **D-Cycloserine Solution:**

**Composition per 10.0mL:**

D-Cycloserine .....	0.2g
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**Preparation of D-Cycloserine Solution:** Add D-cycloserine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Trimethoprim Solution:**

**Composition per 10.0mL:**

Trimethoprim .....	0.025g
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**Preparation of Trimethoprim Solution:** Add trimethoprim to distilled/deionized water and bring volume to 10.0mL. Add a drop of dilute HCl. Mix thoroughly. Gently heat while mixing until dissolved. Filter sterilize.

**Preparation of Medium:** Add components, except cycloheximide solution and tetrazolium chloride solution, D-cycloserine solution, and trimethoprin solution to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Distribute 100.0mL into flasks. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add per 100.0mL of medium, 0.1 mL sterile tetrazolium chloride solution, 0.1mL sterile D-cycloserine solution, 0.1mL sterile trimethoprin solution, and 1.0mL cycloheximide solution. Mix thoroughly. Aseptically pour into sterile Petri dishes.

**Use:** For the selective cultivation of *Agrobacterium tumefaciens* biovar 3.

### *Agrobacterium tumefaciens* Selective Medium

**Composition per 1020mL:**

Agar .....	15.0g
L(–)Arabitol .....	3.04g
K <sub>2</sub> HPO <sub>4</sub> .....	1.04g
KH <sub>2</sub> PO <sub>4</sub> .....	0.54g
Sodium taurocholate .....	0.29g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
NH <sub>4</sub> NO <sub>3</sub> .....	0.16g
Cycloheximide solution .....	10.0mL
Selenite solution .....	10.0mL
Crystal Violet (0.1% solution) .....	2.0mL

#### **Selenite Solution:**

**Composition per 10.0mL:**

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.1g

**Preparation of Selenite Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### **Cycloheximide Solution:**

**Composition per 10.0mL:**

Cycloheximide .....	0.02g
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**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cycloheximide solution and selenite solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute 100.0mL into flasks. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add per 100.0mL of medium, 1.0mL sterile selenite solution and 1.0mL cycloheximide solution. Mix thoroughly. Aseptically pour into sterile Petri dishes.

**Use:** For the selective cultivation of *Agrobacterium tumefaciens* biovar 1.

### *Agrobacterium tumefaciens* Selective Medium

**Composition per 1020mL:**

Agar .....	15.0g
Erythritol .....	3.05g
K <sub>2</sub> HPO <sub>4</sub> .....	1.04g
KH <sub>2</sub> PO <sub>4</sub> .....	0.54g
Sodium taurocholate .....	0.29g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
NH <sub>4</sub> NO <sub>3</sub> .....	0.16g
Cycloheximide solution .....	10.0mL
Selenite solution .....	10.0mL

Malachite Green (0.1% solution) .....	5.0mL
Yeast extract (1% solution) .....	1.0mL

#### **Selenite Solution:**

**Composition per 10.0mL:**

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.1g

**Preparation of Selenite Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### **Cycloheximide Solution:**

**Composition per 10.0mL:**

Cycloheximide .....	0.02g
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**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cycloheximide solution and selenite solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute 100.0mL into flasks. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add per 100.0mL of medium, 1.0mL sterile selenite solution and 1.0mL cycloheximide solution. Mix thoroughly. Aseptically pour into sterile Petri dishes.

**Use:** For the selective cultivation of *Agrobacterium tumefaciens* biovar 2.

### **AKI Medium**

**Composition per liter:**

Peptone .....	15.0g
NaCl .....	5.0g
Yeast extract .....	4.0g
Sodium bicarbonate solution .....	30.0mL
pH 7.2 ± 0.2 at 25°C	

#### **Sodium Bicarbonate Solution:**

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of Sodium Bicarbonate Solution:** Add sodium bicarbonate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Use freshly prepared solution.

**Preparation of Medium:** Add components, except sodium bicarbonate solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile sodium bicarbonate solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Prepare medium freshly.

**Use:** For the cultivation of *Vibrio cholerae* and other *Vibrio* species.

### *Alcaligenes* N5 Medium

**Composition per liter:**

Sodium succinate·2H <sub>2</sub> O .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
NH <sub>4</sub> Cl .....	0.67g
K <sub>2</sub> HPO <sub>4</sub> .....	0.61g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.03g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.0mg
FeCl <sub>3</sub> .....	2.4mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Alcaligenes faecalis*.

***Alcaligenes* NA YE Medium  
(*Alcaligenes* Nutrient Agar  
Yeast Extract Medium)**

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Yeast extract .....	5.0g
Beef extract .....	3.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alcaligenes* species.

***Alcaligenes* NB YE Agar  
(*Alcaligenes* Nutrient Broth  
Yeast Extract Agar)**

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Yeast extract .....	5.0g
Beef extract .....	3.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alcaligenes faecalis*.

***Alcaliphilic Amphibacillus*  
Strains Medium  
(DSMZ Medium 931)**

**Composition** per liter:

Na <sub>2</sub> CO <sub>3</sub> .....	63.6g
NaHCO <sub>3</sub> .....	50.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgCl <sub>2</sub> .....	0.1g
NH <sub>4</sub> Cl .....	0.5g
KCl .....	0.2g
Resazurin .....	0.01g
Sucrose solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Yeast extract solution .....	10.0mL
Vitamin solution .....	10.0mL
Trace elements .....	1.0mL

pH 9.5–10.0 at 25°C

**Sucrose Solution:**

**Composition** per 50.0mL:

Sucrose .....	5.0g
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**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Yeast Extract Solution:**

**Composition** per 10.0mL:

Yeast extract .....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.7g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub>, NH<sub>4</sub>Cl, Na<sub>2</sub>CO<sub>3</sub>, sucrose solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, yeast extract solution, and vitamin solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add solid NaHCO<sub>3</sub>, NH<sub>4</sub>Cl, and Na<sub>2</sub>CO<sub>3</sub>. Mix thoroughly. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium 50.0mL sucrose solution, 10.0mL yeast extract solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solu-

tion, and 10.0mL vitamin solution. The final pH should be 9.5-10.0.

**Use:** For the cultivation of *Amphibacillus fermentum* and *Amphibacillus tropicus*.

### Alginate Utilization Medium

**Composition** per liter:

Solution B	500.0mL
Solution A	400.0mL
Solution C	100.0mL

### Solution A:

**Composition** per 400.0mL:

Marine salts	38.0g
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**Preparation of Solution A:** Add marine salts to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C.

### Solution B:

**Composition** per 500.0mL:

Agar	20.0g
Sodium alginate	10.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C.

### Solution C:

**Composition** per 100.0mL:

Tris-HCl buffer	0.067g
NaNO <sub>3</sub>	0.047g
Ferric EDTA	66.5mg
Sodium glycerophosphate	6.67mg
Thiamine-HCl	67.0µg
Vitamin B <sub>12</sub>	1.3µg
Biotin	0.67µg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine solutions A, B, and C. For liquid medium, omit agar from solution B.

**Use:** For the cultivation of microorganisms that can utilize alginate as a carbon source. Growth on alginate (production of alginase) is a diagnostic test used in the differentiation of *Vibrio* species.

### *Alicyclobacillus acidoterrestris* Agar

**Composition** per 1001.0mL:

Solution A	500.0mL
Solution C	500.0mL
Solution B	1.0mL

pH 4.0 ± 0.2 at 25°C

### Solution A:

**Composition** per 500.0mL:

Glucose	5.0g
KH <sub>2</sub> PO <sub>4</sub>	3.0g
Yeast extract	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
CaCl <sub>2</sub> ·7H <sub>2</sub> O	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0L. Mix thoroughly. Adjust pH to 4.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 50°-55°C.

### Solution B:

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.5g
H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Warm to 50°-55°C.

### Solution C:

**Composition** per 500.0mL:

Agar	15.0g
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**Preparation of Solution C:** Add agar to distilled/deionized water and bring volume to 500.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 50°-55°C.

**Preparation of Medium:** Aseptically combine 500.0mL of solution A, 1.0mL of solution B, and 500.0mL of solution C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Alicyclobacillus acidoterrestris*.

### *Alicyclobacillus* Agar (DSMZ Medium 402)

**Composition** per liter:

Glucose	5.0g
KH <sub>2</sub> PO <sub>4</sub>	3.0g
Yeast extract	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.2g
Agar solution	500.0mL
Trace Elements Solution SL-6	1.0mL

pH 4.0 ± 0.2 at 25°C

### Trace Elements Solution SL-6:

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.5g
H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C. Cool to room temperature.

### Agar Solution:

**Composition** per 500mL:

Agar	15.0g
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**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 45°C.

**Preparation of Medium:** Add components, except trace elements solution SL-6 and agar solution, to distilled/deionized water and bring volume to 499.0mL. Mix thoroughly. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 45°C. Aseptically add 1.0mL of

sterile trace elements solution SL-6 and 500.0mL agar solution. Mix thoroughly. Pour into sterile Petri dishes or aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Alicyclobacillus* spp., *Bacillus* sp., and *Bacillus naganensis*.

#### *Alicyclobacillus cycloheptanicus* Agar (LMG Medium 174)

##### **Composition** per 1001.0mL:

Solution A .....	500.0mL
Agar solution.....	500.0mL
Trace elements solution SL-6 .....	1.0mL
pH 4.0 ± 0.2 at 25°C	

##### **Solution A:**

##### **Composition** per 500.0mL:

Yeast extract .....	5.0g
Glucose .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust to pH 4.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

##### **Trace Elements Solution SL-6:**

##### **Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4. Filter sterilize.

##### **Agar Solution:**

##### **Composition** per 500.0mL:

Agar .....	30.0g
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**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine 500.0mL solution A, 500.0mL sterile agar solution and 1.0mL sterile trace elements solution SL-6. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Alicyclobacillus cycloheptanicus*.

#### **Alkaline *Bacillus* Medium**

##### **Composition** per liter:

Agar .....	15.0g
Peptone.....	10.0g
Glucose .....	10.0g
Yeast extract.....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Na <sub>2</sub> CO <sub>3</sub> solution.....	100.0mL
pH 8.5–11.0 at 25°C	

##### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

##### **Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	10.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Gently heat while stirring and bring to boiling. Autoclave for 15 min at 10 psi pressure–115°C. Cool to 45°–50°C. Aseptically add sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of alkalophilic microorganisms such as *Bacillus alcalophilus*, *Bacillus circulans*, and other *Bacillus* species.

#### **Alkaline Cellulose Agar**

##### **Composition** per liter:

Solution A .....	900.0mL
Solution B.....	100.0mL

##### **Solution A:**

##### **Composition** per 900.0mL:

Agar .....	15.0g
Cellulose powder MN 300 .....	15.0g
NH <sub>4</sub> NO <sub>3</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Peptone .....	1.0g
Yeast extract .....	0.5g
CaCl <sub>2</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 7.0 with 1N HCl. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

##### **Solution B:**

##### **Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	0.5g
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**Preparation of Solution B:** Add 0.5g of Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 9.4 with 6% NaHCO<sub>3</sub> solution. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 900.0mL of solution A with 100.0mL of solution B. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of cellulose-utilizing bacteria.

#### **Alkaline Nutrient Agar**

##### **Composition** per liter:

Agar .....	15.0g
Peptone .....	5.0g
Beef extract .....	3.0g
pH 9.5–10.0 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically adjust to pH 9.5–10.0 with sterile 9% Na<sub>2</sub>CO<sub>3</sub> solution. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus alcalophilus* and *Bacillus* species.

**Alkaline Nutrient Agar****Composition** per liter:

Agar .....	15.0g
Peptone.....	5.0g
NaCl .....	5.0g
Yeast extract.....	2.0g
Beef extract.....	1.0g
Sodium sesquicarbonate solution.....	100.0mL
pH 9.7 ± 0.2 at 25°C	

**Sodium Sesquicarbonate Solution:****Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> , anhydrous.....	10.6g
NaHCO <sub>3</sub> .....	8.42g

**Preparation of Sodium Sesquicarbonate Solution:**

Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 50°–55°C.

**Preparation of Medium:** Add components, except sodium sesquicarbonate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add sterile sodium sesquicarbonate solution. Mix thoroughly. Adjust pH to 9.7. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of alkaliphilic bacteria, including *Bacillus alcalophilus*, *Bacillus cohnii*, and other *Bacillus* species.

**Alkaline Peptone Agar****Composition** per liter:

NaCl .....	20.0g
Agar .....	15.0g
Peptone.....	10.0g
pH 8.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 8.5. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Use:** For the cultivation of *Vibrio cholerae* and other *Vibrio* species.

**Alkaline Peptone Water****Composition** per liter:

Peptone.....	10.0g
NaCl .....	5.0g
pH 8.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.4. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C.

**Use:** For the cultivation of a variety of alkalophilic microorganisms.

**Alkaline Polypectate Agar****Composition** per liter:

Agar .....	16.0g
Na <sub>2</sub> CO <sub>3</sub> .....	10.0g
Peptone.....	6.0g
Sodium polypectate.....	5.0g
Yeast extract.....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
MnSO <sub>4</sub> .....	40.0mg
pH 10.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 10.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of pectinolytic bacteria.

**Alkaline Starch Agar****Composition** per liter:

Starch.....	20.0g
Agar .....	16.0g
Na <sub>2</sub> CO <sub>3</sub> .....	10.0g
Peptone .....	6.0g
Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
MnSO <sub>4</sub> .....	40.0mg
pH 9.7 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 9.7. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of alkaliphilic starch-utilizing bacteria.

**Alkaliphilic Methanogen Medium****Composition** per liter:

NaCl .....	15.0g
NaHCO <sub>3</sub> .....	10.0g
Methanol.....	5.0g
Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> Cl.....	0.5g
Yeast extract .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.0mg
Resazurin .....	0.5mg
Wolfe's mineral solution .....	10.0mL
Selenite-tungstate solution .....	1.0mL
pH 9.2–9.4 at 25°C	

**Wolfe's Mineral Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitilotriacetic acid.....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Selenite-Tungstate Solution:****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Anaerobically distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 9.2–9.4.

**Use:** For the cultivation of *Methanohalophilus zhilinae*.

**Alkaliphilic Spirochete Medium****Composition** per 1011.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	10.0g
NaCl .....	10.0g
NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
KCl .....	0.2g
Yeast extract .....	0.5g
NaHCO <sub>3</sub> solution .....	50.0mL
Sucrose solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Trace elements solution SL-6 .....	1.0mL

pH 9.7 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition** per 50.0mL:

NaHCO <sub>3</sub> .....	15.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Sucrose Solution:****Composition** per 20.0mL:

Sucrose .....	5.0g
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**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Wolfe's Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg

Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Trace Elements Solution SL-6:****Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, sucrose solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 910.0mL. Mix thoroughly. Adjust pH to 9.7 with 6N NaOH (about 15.0mL). Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile sucrose solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Spirochaeta africana*, *Spirochaeta alkalica*, and *Spirochaeta asiatica*.

**Alkaliphilic Sulfur Respiring  
Strains Medium  
(DSMZ Medium 925)**
**Composition** per liter:

Mineral base .....	997.0mL
KSCN solution .....	10.0mL
Trace elements solution .....	2.0mL
Magnesium chloride solution .....	1.0mL

pH 10.0 ± 0.2 at 25°C

**Mineral Base:****Composition** per liter:

Na <sub>2</sub> CO <sub>3</sub> .....	20.0g
NaHCO <sub>3</sub> .....	10.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

**Preparation of Mineral Base:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 20 min at 110°C. The pH should be about 10.0.

**Trace Elements Solution:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	200.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	100.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

EDTA .....	5.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 3.0 with HCl. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Magnesium Chloride Solution:

**Composition per 10.0mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0g
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**Preparation of Magnesium Chloride Solution:** Add MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. A white colloid will dissolve after mixing. Autoclave for 15 min at 15 psi pressure–121°C.

#### KSCN Solution:

**Composition per 10mL:**

KSCN .....	1.5g
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**Preparation of KSCN Solution:** Add KSCN to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Aseptically add 10.0mL sterile KSCN solution, 2.0mL sterile trace elements solution, and 1.0mL sterile magnesium chloride solution to 987.0mL sterile mineral base. Aseptically distribute to sterile tubes, flasks, or bottles.

**Use:** For the cultivation of *Thi alkalivibrio paradoxus* and *Thi alkalivibrio thio cyanoxidans*.

### Alkaliphilic *Thermococcus* Medium (DSMZ Medium 926)

**Composition per 1082.0mL:**

Base solution .....	1000.0mL
Glycine solution .....	50.0mL
Yeast extract solution .....	20.0mL
Polysulfide solution .....	12.0mL

#### Base Solution:

**Composition per 2000.0mL:**

NaCl .....	27.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KCl .....	0.65g
NaHCO <sub>3</sub> .....	0.32g
NaBr .....	0.1g
H <sub>3</sub> BO <sub>3</sub> .....	0.03g
KI .....	15.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
Trace elements solution .....	20.0mL

**Preparation of Base Solution:** Sparge 2.0L of distilled/deionized water with 100% N<sub>2</sub> to remove O<sub>2</sub>. Add components to 2000.0mL of O<sub>2</sub>-free distilled/deionized water. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Do not adjust the pH.

#### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Polysulfide Solution:

**Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.2g
Sulfur .....	0.16g

**Preparation of Polysulfide Solution:** Sparge 100.0mL distilled/deionized water with 100% N<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Add sulfur. The solution will be dark yellow. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Yeast Extract Solution:

**Composition per 100.0mL:**

Yeast extract .....	10.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Glycine Solution:

**Composition per 100.0mL:**

Glycine .....	15.0g
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**Preparation of Glycine Solution:** Add glycine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Aseptically and anaerobically add 50.0mL sterile glycine solution, 20.0mL sterile yeast extract solution, and 12.0mL sterile polysulfide solution to 1.0L sterile base solution. Mix thoroughly. Aseptically and anaerobically distribute to sterile tubes or bottles. Do not adjust pH.

**Use:** For the cultivation of *Thermococcus alcaliphilus*.

### Alkalophile Medium

**Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g
Sodium sesquicarbonate solution .....	15.0mL
pH 9.5 ± 0.2 at 25°C	

#### Sodium Sesquicarbonate Solution:

**Composition per 100.0mL:**

Sodium sesquicarbonate .....	9.0g
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#### Preparation of Sodium Sesquicarbonate Solution:

Add sodium sesquicarbonate to distilled/deionized water and bring volume to 100.0L. Mix thoroughly. Filter sterilize.



**Preparation of Medium:** Add components, except sodium sesquicarbonate solution, to distilled/deionized water and bring volume to 985.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 15.0mL of filter-sterilized sodium sesquicarbonate solution to adjust pH to 9.5. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Bacillus alcalophilus*, *Bacillus circulans*, *Bacillus submarinus*, and other *Bacillus* species.

### Alkalophilic Halophile Agar

**Composition per liter:**

Solution A .....	500.0mL
Solution B .....	500.0mL
pH 9.5 ± 1.0 at 25°C	

#### Solution A:

**Composition per 500.0mL:**

NaCl .....	200.0g
Na <sub>2</sub> CO <sub>3</sub> ·10H <sub>2</sub> O .....	50.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Solution B:

**Composition per liter:**

Agar .....	20.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Trisodium citrate .....	3.0g
KCl .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.36mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 500.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 500.0mL of solution A with 500.0mL of solution B. Mix thoroughly. Bring pH to 9.5. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Natronobacterium gregoryi*, *Natronobacterium magadii*, *Natronobacterium pharaonis*, *Natronobacterium vacuolata*, and *Natronococcus occultus*.

### Allen and Arnon Medium with Nitrate

**Composition per 1000.25mL:**

Noble agar .....	10.0g
KNO <sub>3</sub> .....	0.253g
NaNO <sub>3</sub> .....	0.212g
Solution A .....	25.0mL
Solution B .....	6.25mL

#### Solution A:

**Composition per 2.0L:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O (4% solution) .....	500.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O (1.2% solution) .....	500.0mL
NaCl (3.8% solution) .....	500.0mL
Microelements stock solution .....	500.0mL

**Preparation of Solution A:** Prepare individual solutions and combine.

### Microelements Stock Solution:

**Composition per 1090.0mL:**

H <sub>3</sub> BO <sub>3</sub> .....	572.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	360.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	44.0mg
MoO <sub>3</sub> .....	36.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	15.8mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	8.0mg
NH <sub>4</sub> VO <sub>3</sub> .....	4.6mg
A & A FeEDTA solution .....	160.0mL

**Preparation of Microelements Stock Solution:** Add components to distilled/deionized water and bring volume to 1090.0mL. Mix well.

### A & A FeEDTA Solution:

**Composition per 550.0mL:**

Disodium EDTA·2H <sub>2</sub> O .....	20.4g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	13.7g
KOH .....	5.2g

**Preparation of A & A FeEDTA Solution:** Dissolve 5.2g of KOH in 186.0mL of distilled/deionized water. Add 20.4g of disodium EDTA·2H<sub>2</sub>O. Add 13.7g of FeSO<sub>4</sub>·7H<sub>2</sub>O to 364.0mL of distilled/deionized water. Combine the EDTA solution with the FeSO<sub>4</sub> solution. Sparge solution with filtered air until color changes. pH about 3.5.

#### Solution B:

**Composition per 500.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	28.0g
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**Preparation of Solution B:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 500.0mL.

**Preparation of Medium:** Add agar, KNO<sub>3</sub>, and NaNO<sub>3</sub> to distilled/deionized water and bring volume to 969.0mL. Mix thoroughly. Gently heat and bring to boiling. Add 25.0mL of solution A. Autoclave for 15 min at 15 psi pressure–121°C. Add 6.25mL of solution B aseptically after sterilization.

**Use:** For the cultivation and maintenance of *Anabaena* species and *Nostoc* species.

### AMB Agar

**Composition per liter:**

Agar .....	15.0g
Starch, soluble .....	5.0g
Pancreatic digest of casein .....	2.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

### AMB Broth

**Composition per liter:**

Starch, soluble .....	5.0g
Pancreatic digest of casein .....	2.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of myxobacteria.

**Amino-butyric Acid Medium****Composition per liter:**

Agar .....	15.0g
DL-Amino-butyric acid .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	7.0g
Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and maintenance of *Serratia marcescens* and other microorganisms that can utilize amino-butyric acid as a carbon source.

**Ammonifex Medium****Composition per 1010.0mL:**

NaCl .....	1.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.22g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.22g
NaHCO <sub>3</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.09g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2mg
Resazurin .....	0.5mg
KNO <sub>3</sub> solution .....	10.0mL
Selenite-tungstate solution .....	3.0mL
Wolfe's mineral solution .....	1.0mL

pH 5.4 ± 0.2 at 25°C

**Selenite-Tungstate Solution:****Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**KNO<sub>3</sub> Solution:****Composition per 100.0mL:**

KNO <sub>3</sub> .....	10.0g
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**Preparation of KNO<sub>3</sub> Solution:** Add KNO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O and KNO<sub>3</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Adjust pH to 6.5 with 20% HCl. Sparge with 100% N<sub>2</sub> for 30 min. Dispense anaerobically into tubes in 10.0mL aliquots. Evacuate headspace under vacuum. Pressurize tubes to 200 kPa with 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 5.4. Prior to use, aseptically and anaerobically add 0.1mL of sterile KNO<sub>3</sub> to each tube.

**Use:** For the cultivation of *Ammonifex* species that form ammonium from nitrate during chemolithoautotrophic growth of this extremely thermophilic bacterium.

**AMS Agar****(Ammonium Mineral Salts Agar)****Composition per liter:**

Agar .....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
KH <sub>2</sub> PO <sub>4</sub> .....	0.54g
NH <sub>4</sub> Cl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0mg
H <sub>3</sub> BO <sub>3</sub> .....	0.3mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Add sterile methanol to a concentration of 0.5% aseptically to cooled basal medium.

**Use:** For the cultivation and maintenance of bacteria that can utilize methanol as a carbon source, such as *Methylobacterium* species, *Methylomonas* species, and *Methylophilus* species.

**AMS Agar without Methanol****(Ammonium Mineral Salts  
Agar without Methanol)****Composition per liter:**

Agar .....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
KH <sub>2</sub> PO <sub>4</sub> .....	0.54g
NH <sub>4</sub> Cl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0mg
H <sub>3</sub> BO <sub>3</sub> .....	0.3mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03mg

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01mg
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methylosinus trichosporium* and other methane-oxidizing bacteria. Cultures are grown under an atmosphere of 50% methane.

#### AN1 Medium

##### Composition per liter:

Pancreatic digest of casein .....	10.0g
Sulfur, powdered .....	8.0g
NaCl .....	2.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
Disodium thioglycolate solution .....	1.0g
Resazurin .....	1.0mg
pH 7.3 ± 0.2 at 25°C	

#### Disodium Thioglycolate Solution:

##### Composition per 10.0mL:

Disodium thioglycolate .....	1.0g
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**Preparation of Disodium Thioglycolate Solution:** Add disodium thioglycolate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except powdered sulfur and disodium thioglycolate solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Allow to cool under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Powdered sulfur is sterilized separately by steaming for 3 hr on 3 consecutive days. Aseptically and anaerobically combine the basal solution, sterile powdered sulfur, and sterile disodium thioglycolate solution under 100% N<sub>2</sub>.

**Use:** For the cultivation of *Thermococcus* species.

#### Anacker and Ordal Medium

##### Composition per liter:

Agar .....	10.0g
Pancreatic digest of casein .....	0.5g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g
Beef extract .....	0.2g
pH 7.3 ± 0.1 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flexibacter columnaris*.

#### Anacker and Ordal Medium, Enriched

##### Composition per liter:

Agar .....	10.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g
Beef extract .....	0.2g
pH 7.3 ± 0.1 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flexibacter psychrophilus*.

#### Anaerobaculum thermoterrenum Medium (DSMZ Medium 104a)

##### Composition per liter:

Yeast extract .....	10.0g
NaCl .....	8.0g
Trypticase peptone .....	5.0g
Peptone .....	5.0g
Beef extract .....	5.0g
Glucose .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
L-Cysteine·HCl .....	0.5g
Resazurin .....	1.0mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	50.0mL
Salt solution .....	40.0mL
Glucose solution .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

#### Salt Solution:

##### Composition per liter:

NaHCO <sub>3</sub> .....	10.0g
NaCl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	5.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Glucose Solution:

##### Composition per 100.0mL:

D-Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add components, except L-cysteine, glucose solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Sparge with CO<sub>2</sub>. Add L-cysteine. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 50.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 10.0mL sterile glucose solution. Mix thoroughly. Adjust pH to 7.0 with 8N NaOH. Distribute into sterile tubes or flasks under anaerobic N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Anaerobaculum thermoterrenum*.

#### Anaerobic Agar

##### Composition per liter:

Pancreatic digest of casein .....	20.0g
Agar .....	15.0g
Yeast extract .....	15.0g

NaCl .....	5.0g
Sodium thioglycolate .....	2.0g
Sodium formaldehyde sulfoxylate .....	1.0g
pH $7.2 \pm 0.2$ at $25^{\circ}\text{C}$	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes until medium is 3 inches deep. Autoclave for 20 min at 15 psi pressure– $121^{\circ}\text{C}$ .

**Use:** For the anaerobic cultivation of *Bacillus* species, especially *Bacillus larvae*, *Bacillus popilliae*, and *Bacillus lentimorbus*.

#### Anaerobic Cellulolytic Medium

##### Composition per liter:

$\text{NH}_4\text{Cl}$ .....	1.0g
Cellobiose .....	1.0g
Yeast extract .....	1.0g
$\text{MgSO}_4$ .....	0.5g
KCl .....	0.5g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.5g
$\text{K}_2\text{HPO}_4$ .....	0.4g
Resazurin .....	1.0mg
Wolfe's mineral solution .....	20.0mL
$\text{Na}_2\text{CO}_3$ solution .....	10.0mL
$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ solution .....	10.0mL
pH $6.9 \pm 0.1$ at $25^{\circ}\text{C}$	

##### Wolfe's Mineral Solution:

###### Composition per liter

$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
$\text{MnSO}_4\cdot \text{H}_2\text{O}$ .....	0.5g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.1g
$\text{CaCl}_2$ .....	0.1g
$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ .....	0.01g
$\text{AlK}(\text{SO}_4)_2\cdot 12\text{H}_2\text{O}$ .....	0.01g
$\text{H}_3\text{BO}_3$ .....	0.01g
$\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to approximately 500.0mL of distilled/deionized water and adjust pH to 6.5 with KOH to dissolve. Bring volume to 1.0L with distilled/deionized water. Add other compounds. Mix thoroughly.

##### $\text{Na}_2\text{CO}_3$ Solution:

###### Composition per 100.0mL:

$\text{Na}_2\text{CO}_3$ .....	10.0g
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**Preparation of  $\text{Na}_2\text{CO}_3$  Solution:** Add  $\text{Na}_2\text{CO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

##### $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ Solution:

###### Composition per 100.0mL:

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ .....	15.0g
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**Preparation of  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except  $\text{Na}_2\text{CO}_3$  solution and  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, to distilled/deionized water and bring volume to 980.0mL. Boil medium under 80%  $\text{N}_2$  + 10%  $\text{CO}_2$  + 10%  $\text{H}_2$  until medium is colorless. Cool and distribute anaerobically into test tubes in 10.0mL volumes using 80%  $\text{N}_2$  + 10%  $\text{CO}_2$  + 10%  $\text{H}_2$ .

Stopper the tubes anaerobically. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ . Cool to room temperature. Aseptically add 0.1mL of sterile  $\text{Na}_2\text{CO}_3$  solution and 0.1mL of sterile  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution to each tube. Mix thoroughly.

**Use:** For the cultivation and maintenance of microorganisms that can utilize cellobiose as sole carbon source, such as *Clostridium cellulovorans*.

#### Anaerobic Citrate Medium

##### Composition per liter:

Ferric citrate .....	17.0g
Sodium acetate .....	6.8g
$\text{NaHCO}_3$ .....	2.5g
$\text{NH}_4\text{Cl}$ .....	1.5g
$\text{NaH}_2\text{PO}_4\cdot \text{H}_2\text{O}$ .....	0.6g
KCl .....	0.1g
Trace elements solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
pH $7.0 \pm 0.2$ at $25^{\circ}\text{C}$	

##### Trace Elements Solution:

###### Composition per liter:

$\text{Na}_2\text{WO}_4$ .....	25.0mg
$\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ .....	24.0mg
Wolfe's mineral solution .....	1.0L

##### Wolfe's Mineral Solution:

###### Composition per liter:

$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
$\text{MnSO}_4\cdot \text{H}_2\text{O}$ .....	0.5g
$\text{CaCl}_2$ .....	0.1g
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.1g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{AlK}(\text{SO}_4)_2\cdot 12\text{H}_2\text{O}$ .....	0.01g
$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ .....	0.01g
$\text{H}_3\text{BO}_3$ .....	0.01g
$\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to approximately 500.0mL of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0L with remaining water and add remaining compounds one at a time.

**Preparation of Trace Elements Solution:** Combine components. Mix thoroughly.

##### Wolfe's Vitamin Solution:

###### Composition per liter:

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Add ferric citrate to 500.0mL of boiling distilled/deionized water. Mix thoroughly. Cool to room temperature while sparging with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ .

Add remaining components. Add distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with NaOH. Continue sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Geobacter metallireducens*.

***Anaerobranca gottschalkii* Medium  
(DSMZ Medium 895)**

**Composition** per 1020mL:

NaCl .....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
L-Cysteine .....	0.5g
NH <sub>4</sub> Cl .....	0.4g
Yeast extract .....	0.25g
Tryptone .....	0.25g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	0.5mg
Na <sub>2</sub> CO <sub>3</sub> solution .....	50.0mL
Soluble starch solution .....	20.0mL
Trace elements solution .....	10.0mL
Vitamin solution, 10 fold conc. ....	1.0mL
pH 9.4 ± 0.2 at 25°C	

**Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:**

**Composition** per 100mL:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>CO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Starch Solution:**

**Composition** per 50.0mL:

Starch, soluble .....	5.0g
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**Preparation of Starch Solution:** Add starch to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except starch solution, Na<sub>2</sub>CO<sub>3</sub> solution, and L-cysteine, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to 25°C while sparging with 100% N<sub>2</sub>. Add 0.5g L-cysteine. Mix thoroughly. Distribute to anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add, per liter of medium, 20.0mL sterile starch solution, and 50.0mL sterile Na<sub>2</sub>CO<sub>3</sub> solution. Final pH is 9.3–9.5.

**Use:** For the cultivation of *Anaerobranca gottschalkii*, a novel thermoalkaliphilic bacterium that grows anaerobically at high pH and temperature.

***Anaerobranca* Medium**

**Composition** per 1015.0mL:

Yeast extract .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	3.9g
Sodium fumarate .....	1.5g
KCl .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.125g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.125g
Wolfe's vitamin solution .....	10.0mL
Wolfe's mineral solution .....	5.0mL
pH 8.5 ± 0.2 at 25°C	

**Wolfe's Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Wolfe's Mineral Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Wolfe's vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 8.5. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Anaerobranca horikoshii*.

#### Anaerocellum Medium

##### Composition per liter:

Cellobiose or starch .....	5.0g
NaHCO <sub>3</sub> .....	1.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
NH <sub>4</sub> Cl .....	0.33g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	100.0mL
Cellobiose or starch solution .....	50.0mL
Vitamin solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.1–7.3 at 25°C	

##### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

##### Cellobiose or Starch Solution:

##### Composition per 50.0mL:

Cellobiose or starch .....	5.0g
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**Preparation of Cellobiose or Starch Solution:** Add cellobiose or starch to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Gas under 100% N<sub>2</sub>.

##### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

##### Vitamin Solution:

##### Composition per liter:

Pyridoxine·HCl .....	10.0mg
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Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, cellobiose or starch solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 830.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add sterile NaHCO<sub>3</sub> solution, sterile cellobiose or starch solution, and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Anaerocellum thermophilum* and *Dictyoglossus turgidus*.

#### Anaerocellum Medium

##### Composition per liter:

NaHCO <sub>3</sub> .....	1.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
NH <sub>4</sub> Cl .....	0.33g
Yeast extract .....	0.2g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	100.0mL
Cellobiose or starch solution .....	50.0mL
Vitamin solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.1–7.3 at 25°C	

##### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Cellobiose or Starch Solution:****Composition** per 50.0mL:

Cellobiose or starch ..... 5.0g

**Preparation of Cellobiose or Starch Solution:** Add cellobiose or starch to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Gas under 100% N<sub>2</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition** per liter:

Pyridoxine·HCl ..... 10.0mg  
 Calcium DL-pantothenate ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Thiamine·HCl ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
 HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, cellobiose or starch solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 830.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add sterile NaHCO<sub>3</sub> solution, sterile cellobiose or starch solution, and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Anaerocellum thermophilum* and *Dictyoglomus turgidus*.

***Anaerovibrio burkinabensis* Medium****Composition** per 1001.0mL:

Solution A ..... 870.0mL  
 Solution C ..... 100.0mL  
 Solution D ..... 10.0mL  
 Solution E (Vitamin solution) ..... 10.0mL

Solution F ..... 10.0mL  
 Solution G ..... 10.0mL  
 Solution B (Trace elements solution SL-10) ..... 1.0mL  
 pH 6.8–7.2 at 25°C

**Solution A:****Composition** per 870.0mL:

Na<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
 NaCl ..... 1.0g  
 KCl ..... 0.5g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.4g  
 NH<sub>4</sub>Cl ..... 0.3g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
 Resazurin ..... 1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
 HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:NaHCO<sub>3</sub> ..... 5.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition** per 10.0mL:

Sodium lactate ..... 2.5g

**Preparation of Solution D:** Add sodium lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition** per liter:

Pyridoxine·HCl ..... 10.0mg  
 Calcium DL-pantothenate ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Thiamine·HCl ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution G:

**Composition per 10.0mL:**

Yeast extract ..... 1.0g

**Preparation of Solution F:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, solution F, and solution G, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Anaerovibrio burkinabensis*, a lactate-fermenting bacterium isolated from rice field soils.

#### *Ancylobacter-Spirosoma* Agar

**Composition per liter:**

Agar ..... 20.0g

Glucose ..... 1.0g

Peptone ..... 1.0g

Yeast extract ..... 1.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Ancylobacter aquaticus*, *Ancylobacter* species, *Aquaspirillum metamorphum*, *Aquaspirillum serpens*, *Flectobacillus major*, *Methylobacterium mesophilicum*, *Runella slithyiformis*, *Shewanella putrefaciens*, and *Spirosoma linguale*.

#### *Ancylobacter-Spirosoma* Medium (DSMZ Medium 7)

**Composition per liter:**

Agar ..... 15.0g

Glucose ..... 1.0g

Peptone ..... 1.0g

Yeast extract ..... 1.0g

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Spirosoma linguale*.

#### Anderson's Marine Agar

**Composition per liter:**

Agar ..... 15.0g

Peptone ..... 2.5g

Yeast extract ..... 2.5g

FePO<sub>4</sub> ..... 0.1g

Filtered, aged seawater ..... 750.0mL

pH 7.4–7.6 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.4–7.6. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Vibrio* species.

#### Anderson's Marine Broth

**Composition per liter:**

Peptone ..... 2.5g

Yeast extract ..... 2.5g

FePO<sub>4</sub> ..... 0.1g

Filtered, aged seawater ..... 750.0mL

pH 7.4–7.6 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4–7.6. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Vibrio* species.

#### Anderson's Marine Medium

**Composition per liter:**

Peptone ..... 2.5g

Yeast extract ..... 2.5g

FePO<sub>4</sub> ..... 0.1g

Filtered, aged seawater ..... 750.0mL

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except seawater, to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 750.0mL of sterile aged seawater. Mix thoroughly. Bring pH to 7.4. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Flavobacterium* species, *Micrococcus* species, *Planococcus* species, *Pseudomonas fluorescens*, and *Vibrio* species.

#### Andrade's Broth

**Composition per liter:**

Pancreatic digest of gelatin ..... 10.0g

NaCl ..... 5.0g

Beef extract ..... 3.0g

Andrade's indicator ..... 10.0mL

Carbohydrate solution ..... 50.0mL

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a prepared medium from BD Diagnostic Systems, in tubes containing adonitol, arabinose, cellobiose, glucose, dulcitol, fructose, galactose, inositol, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, or xylose.

#### Andrade's Indicator

**Composition per 100.0mL:**

NaOH (1N solution) ..... 16.0mL

Acid Fuchsin ..... 0.1g

**Preparation of Andrade's Indicator:** Add Acid Fuchsin to NaOH solution and bring volume to 100.0mL with distilled/deionized water.



**Carbohydrate Solution:****Composition** per 100.0mL:

Carbohydrate..... 10.0g

**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 100.0mL. Adonitol, arabinose, cellobiose, glucose, dulcitol, fructose, galactose, inositol, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, xylose, or other carbohydrates may be used. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute in 10.0mL volumes into test tubes containing inverted Durham tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Add 0.5mL of sterile carbohydrate solution to each tube.

**Caution:** Acid Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Use:** For the determination of carbohydrate fermentation reactions of microorganisms, particularly members of the Enterobacteriaceae. A Durham tube is used to collect gas produced during the fermentation reaction. Acid production is indicated by a pink color.

**AO Agar****Composition** per liter:

Agar ..... 11.0g  
Sodium acetate ..... 0.5g  
Pancreatic digest of casein ..... 0.5g  
Yeast extract ..... 0.5g  
Beef extract ..... 0.2g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

**Aolpha Medium****Composition** per 1041.0mL:

NaCl ..... 100.0g  
Agar ..... 15.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 9.5g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.0g  
KCl ..... 5.0g  
Peptone ..... 5.0g  
Yeast extract ..... 1.0g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.2g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.1g  
KNO<sub>3</sub> ..... 0.1g  
Metals solution ..... 20.0mL  
Phosphate solution ..... 20.0mL  
Vitamin solution ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

**Metals Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 29.7g  
Nitrilotriacetic acid ..... 10.0g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 3.3g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 99.0mg

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 12.7mg  
“Metals 44” ..... 50.0mL

**Preparation of Metals Solution:** Solubilize nitrilotriacetic acid with KOH. Dissolve remaining ingredients. Adjust pH to 7.2 with KOH or H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile basal medium.

**“Metals 44”:****Composition** per 100.0mL:

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
EDTA ..... 0.25g  
MnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.154g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.04g  
Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O ..... 0.018g

**Preparation of “Metals 44”:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile basal medium.

**Phosphate Solution:****Composition** per liter:

K<sub>2</sub>HPO<sub>4</sub> ..... 2.5g  
KH<sub>2</sub>PO<sub>4</sub> ..... 2.5g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile basal medium.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Calcium pantothenate ..... 5.0mg  
Nicotinamide ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize and add aseptically to sterile basal medium.

**Preparation of Medium:** Add components—except “Metals 44”, phosphate solution, and vitamin solution—to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH of basal medium to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C and aseptically add the “Metals 44”, phosphate, and vitamin solutions.

**Use:** For the cultivation and maintenance of *Halomonas meridiana* and other *Halomonas* species.

**Aplanobacterium Medium****Composition** per liter:

Agar ..... 20.0g  
Glucose ..... 10.0g  
Peptone ..... 5.0g  
Yeast extract ..... 5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15

psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas* species.

### Apple Juice Yeast Extract Medium (AJYE Medium)

**Composition** per 1200.0mL:

Agar .....	30.0g
Yeast extract .....	10.0g
Apple juice .....	1.0L

pH 4.8 ± 0.2 at 25°C

**Preparation of Medium:** Add yeast extract to 1.0L of apple juice. Mix thoroughly. Adjust pH to 4.8. Autoclave for 10 min at 9 psi pressure–114°C. Cool to 45°–50°C. In a separate flask, add agar to 200.0mL of distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine the sterile apple juice solution with the sterile agar solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the cultivation of *Zymomonas* species.

### *Aquabacter spiritensis* Medium (LMG Medium 225)

Sodium succinate .....	2.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
Peptone .....	0.4g
NH <sub>4</sub> Cl .....	0.2g
NaCl .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Ferric citrate .....	5.0mg
Vitamin solution .....	20.0mL
SL-6 Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

### SL-6 Trace Elements Solution:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of SL-6 Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

### Vitamin Solution:

**Composition** per liter:

Calcium DL-pantothenate .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to 980.0mL distilled/deionized water. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 20.0mL sterile vitamin solu-

tion. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Aquabacter spiritensis*.

### *Aquaspirillum* Autotrophic Agar

**Composition** per liter:

Noble agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate .....	5.0mg
NaHCO <sub>3</sub> solution .....	10.0mL
Trace elements solution .....	3.0mL

pH 7.1 ± 0.2 at 25°C

### NaHCO<sub>3</sub> Solution:

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	0.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

### Trace Elements Solution:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	30.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	3.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to double-distilled water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes. For autotrophic growth, incubate under 85% H<sub>2</sub> + 10% CO<sub>2</sub> + 5% O<sub>2</sub>.

**Use:** For the autotrophic cultivation and maintenance of *Aquaspirillum autotrophicum*.

### *Aquaspirillum* Heterotrophic Agar

**Composition** per liter:

Noble agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
Sodium succinate .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate .....	5.0mg
Trace elements solution .....	3.0mL

pH 7.1 ± 0.2 at 25°C

### Trace Elements Solution:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	30.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	3.0mg

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to double-distilled water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the heterotrophic cultivation and maintenance of *Aquaspirillum autotrophicum*.

### *Aquaspirillum Medium* (DSMZ Medium 888)

#### Composition per 1026mL:

Casamino acids .....	1.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Agar .....	0.5g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> .....	10.0mg
Sodium succinate solution .....	10.0mL
Thiosulfate solution .....	10.0mL
Standard vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Standard Vitamin Solution:

##### Composition per 100mL:

Thiamine-HCl·2H <sub>2</sub> O .....	50.0mg
Nicotinic acid .....	50.0mg
Pyridoxine-HCl .....	50.0mg
Ca-pantothenate .....	50.0mg
Riboflavin .....	10.0mg
Vitamin B <sub>12</sub> .....	1.0mg
Folic acid .....	0.2mg
Biotin .....	0.1mg

**Preparation of Standard Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Thiosulfate Solution:

##### Composition per 10mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	1.0g
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**Preparation of Thiosulfate Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Sodium Succinate Solution:

##### Composition per 10mL:

Sodium succinate .....	1.0g
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**Preparation of Sodium Succinate Solution:** Add sodium succinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except sodium succinate solution, thiosulfate solution, standard vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL sterile sodium succinate solution, 1.0mL sterile thiosulfate solution, 5.0mL sterile standard vitamin solution, and 1.0mL sterile trace elements solution SL-10. Mix thoroughly. Adjust pH to 7.5. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Aquaspirillum* spp.

### *Archaeoglobus Medium*

#### Composition per liter:

NaCl .....	18.0g
NaHCO <sub>3</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.45g
Sodium L-lactate .....	1.5g
Yeast extract .....	0.5g
KCl .....	0.34g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	25.0mL
Trace elements solution .....	10.0mL

pH 6.9 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 50.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Prepare and dispense solution anaerobically under with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with

additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**Preparation of Medium:** Add components, except  $\text{NaHCO}_3$  and  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, to distilled/deionized water and bring volume to 1.0L. Mix well and heat to boiling for a few min. Cool rapidly to room temperature while gassing with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Add  $\text{NaHCO}_3$  and adjust pH to 6.9. Distribute anaerobically under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  and pressurize sealed containers up to 2 bar pressure. Autoclave for 15 min at 15 psi pressure–121°C. Prior to inoculation of cultures, add 0.25mL of sterile  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution to each tube containing 9.75mL of sterile basal medium.

**Use:** For the cultivation and maintenance of *Archaeoglobus fulgidus*.

#### *Archaeoglobus profundus* Medium

**Composition per liter:**

NaCl .....	18.0g
$\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ .....	4.0g
$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ .....	3.45g
$\text{Na}_2\text{SO}_4$ .....	2.7g
Sodium acetate .....	1.0g
$\text{NaHCO}_3$ .....	1.0g
Yeast extract .....	0.5g
KCl .....	0.34g
$\text{NH}_4\text{Cl}$ .....	0.25g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.14g
$\text{K}_2\text{HPO}_4$ .....	0.14g
$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2\cdot 7\text{H}_2\text{O}$ .....	2.0mg
Resazurin .....	1.0mg
$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ solution .....	25.0mL
Trace elements solution .....	10.0mL

pH  $6.5 \pm 0.2$  at 25°C

#### **Trace Elements Solution:**

**Composition per liter:**

$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
$\text{MnSO}_4\cdot 2\text{H}_2\text{O}$ .....	0.5g
$\text{CoSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.18g
$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.18g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.1g
$\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.025g
$\text{KAl}(\text{SO}_4)_2\cdot 12\text{H}_2\text{O}$ .....	0.02g
$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ .....	0.01g
$\text{H}_3\text{BO}_3$ .....	0.01g
$\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ .....	0.01g
$\text{Na}_2\text{SeO}_3\cdot 5\text{H}_2\text{O}$ .....	0.3mg

#### **$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ Solution:**

**Composition per 50.0mL:**

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ .....	1.0g
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**Preparation of  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  Solution:** Prepare and dispense solution anaerobically under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**Preparation of Medium:** Add components, except  $\text{NaHCO}_3$  and  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, to distilled/deionized water and bring volume to 1.0L. Mix well and heat to boiling for a few min. Cool rapidly to room temperature while gassing with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Add  $\text{NaHCO}_3$  and adjust pH to 6.9. Distribute anaerobically under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  and pressurize sealed containers up to 2 bar pressure. Autoclave for 15 min at 15 psi pressure–121°C. Prior to inoculation of cultures, add 0.25mL of sterile  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution to each tube containing 9.75mL of sterile basal medium.

**Use:** For the cultivation and maintenance of *Archaeoglobus profundus*.

#### *Archaeoglobus veneficus* Medium (DSMZ Medium 796)

**Composition per liter:**

NaCl .....	18.0g
$\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ .....	7.15g
$\text{NaHCO}_3$ .....	5.0g
KCl .....	0.33g
$\text{NH}_4\text{Cl}$ .....	0.25g
$\text{K}_2\text{HPO}_4\cdot 3\text{H}_2\text{O}$ .....	0.18g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.14g
$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2\cdot 6\text{H}_2\text{O}$ .....	2.0mg
Resazurin .....	0.5mg
$\text{Na}_2\text{SO}_3$ solution .....	20.0mL
$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ solution .....	10.0mL
Na-acetate solution .....	4.0mL
Trace elements solution .....	1.0mL

pH  $1.0 \pm 0.2$  at 25°C

#### **$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ Solution:**

**Composition per 20.0mL:**

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ .....	0.5g
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**Preparation of  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 20.0mL. Sparge with  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### **$\text{Na}_2\text{SO}_3$ Solution:**

**Composition per 10.0mL:**

$\text{Na}_2\text{SO}_3$ .....	0.5g
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**Preparation of  $\text{Na}_2\text{SO}_3$  Solution:** Add  $\text{Na}_2\text{SO}_3$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Filter sterilize.

#### **Na-acetate Solution:**

**Composition per 10.0mL:**

Na-acetate .....	2.5g
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**Preparation of Na-acetate Solution:** Add Na-acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Filter sterilize.

#### **Trace Element Solution:**

**Composition per liter:**

NaCl .....	5.0g
$\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ .....	2.9g
$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2$ .....	1.0g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.5g
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.5g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.5g
$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.5g
$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ .....	0.05g
$\text{H}_3\text{BO}_3$ .....	0.05g
$\text{KAl}(\text{SO}_4)_2\cdot 12\text{H}_2\text{O}$ .....	0.05g
$\text{Na}_2\text{MO}_4\cdot 4\text{H}_2\text{O}$ .....	0.05g

Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.05g

**Preparation of Trace Element Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except Na-acetate solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and NaHCO<sub>3</sub>, to 986.0mL distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add the solid NaHCO<sub>3</sub>. Equilibrate with the 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas. Adjust pH to 7.0 with HCl. Add 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Distribute into serum bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas; 25.0mL medium in 100mL serum bottles. Adjust pH to 1.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically inject 0.1mL sterile Na-acetate solution and 0.5mL Na<sub>2</sub>SO<sub>3</sub> solution per 25.0mL medium. Mix thoroughly. After inoculation pressurize to 1 bar atmosphere with 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas.

**Use:** For the cultivation of *Archaeoglobus veneficus*.

#### *Archangium violaceum* Medium

##### Composition per liter:

Monosodium glutamate .....	1.0g
L-Leucine .....	0.5g
L-Tyrosine .....	0.5g
L-Isoleucine .....	0.3g
L-Proline .....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
L-Lysine .....	0.15g
L-Arginine .....	0.1g
L-Asparagine .....	0.1g
L-Serine .....	0.1g
L-Threonine .....	0.1g
L-Valine .....	0.1g
L-Alanine .....	0.05g
L-Glycine .....	0.05g
L-Histidine .....	0.05g
L-Methionine .....	0.05g
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	0.02g
KCl .....	0.02g
Tris(hydroxymethyl)aminomethane buffer (0.02m solution, pH 7.5) .....	1.0L
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add solid components to 1.0L of Tris buffer. Mix thoroughly. Filter sterilize. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation of *Archangium violaceum*.

#### Artificial Deep Lake Medium

##### Composition per liter:

NaCl .....	180.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	75.0g
Noble agar .....	15.0g
Sodium succinate .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.4g
KCl .....	7.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
Yeast extract .....	1.0g
Vitamin solution .....	10.0mL
pH 7.4 ± 0.2 at 25°C	

##### Vitamin Solution:

##### Composition per liter:

Biotin .....	30.0mg
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Cyanocobalamin .....	20.0mg
Thiamine-HCl .....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize and add aseptically to sterile basal medium.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust medium to pH 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL of vitamin solution. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Halobacterium lacusprofundi*.

#### Artificial Organic Lake Peptone Medium

##### Composition per 1001.0mL:

NaCl .....	30.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.5g
KCl .....	5.0g
Peptone .....	5.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
KNO <sub>3</sub> .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
Modified Hutner's basal salts .....	20.0mL
Phosphate supplement .....	20.0mL
Artificial organic lake vitamin solution .....	1.0mL
pH 7.3 ± 0.2 at 25°C	

##### Modified Hutner's Basal Salts:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitritotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
Ammonium molybdate .....	9.25mg
"Metals 44" .....	50.0mL

##### Preparation of Modified Hutner's Basal Salts:

Dissolve the nitritotriacetic acid first and neutralize the solution with KOH. Add other components and adjust the pH to 7.2 with KOH or H<sub>2</sub>SO<sub>4</sub>. There may be a slight precipitate. Store at 5°C.

##### "Metals 44":

##### Composition per liter:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile modified Hutner's basal salts solution.

##### Phosphate Supplement:

##### Composition per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	2.5g

**Preparation of Phosphate Supplement:** Add components to distilled/deionized water and bring volume to

20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Artificial Organic Lake Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Nicotinamide .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Artificial Organic Lake Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Store at 5°C.

**Preparation of Medium:** Add components, except modified Hutner's basal salts solution, phosphate supplement solution, and vitamin solution, to distilled/deionized water and bring volume to 959.0mL. Mix thoroughly. Bring pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 20.0mL of sterile modified Hutner's basal salts solution, 20.0mL of sterile phosphate supplement solution, and 1.0mL of sterile artificial organic lake vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Halomonas meridiana*.

#### Ashbey's Nitrogen-Free Agar

##### Composition per liter:

Agar .....	15.0g
Mannitol .....	15.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
MoO <sub>3</sub> (10% solution) .....	0.1mL
FeCl <sub>3</sub> (10% solution) .....	0.05mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of bacteria, such as *Azotobacter* species and cyanobacteria, that can utilize atmospheric N<sub>2</sub> as sole nitrogen source.

#### ASN-III Agar

##### Composition per liter:

NaCl .....	25.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0g
NaNO <sub>3</sub> .....	0.75g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.75g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
KCl .....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	3.0mg
Ferric ammonium citrate .....	3.0mg
Magnesium EDTA .....	0.5mg
Vitamin B <sub>12</sub> .....	10.0μg
Agar solution .....	100.0mL
A-5 trace metals .....	1.0mL

pH 7.3 ± 0.2 at 25°C

#### Agar Solution:

##### Composition per 100.0mL:

Noble agar .....	10.0g
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**Preparation of Agar Solution:** Add agar to glass distilled water and bring volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### A-5 Trace Metals:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.039g

**Preparation of A-5 Trace Metals:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except agar solution, to glass distilled water and bring volume to 900.0mL. Mix well and heat gently until dissolved. Filter sterilize. Warm to 45°–50°C. Aseptically add agar solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Xenococcus* species. For the isolation of cyanobacteria from marine habitats.

#### Asparagine Broth

##### Composition per liter:

DL-Asparagine .....	30.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

pH 6.9–7.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix well until dissolved. Adjust pH to between 6.9 and 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For a presumptive test medium in the differentiation of nonfermentative Gram-negative bacteria, especially *Pseudomonas aeruginosa*. For use in the multiple-tube technique in the microbiological analysis of recreational waters.

#### Asticcacaulis Medium

##### Composition per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	0.5g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Asticcacaulis* species.

#### ASTM Nutrient Salts Agar (American Society for Testing and Materials Nutrient Salts Agar)

##### Composition per liter:

Agar .....	15.0g
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KH <sub>2</sub> PO <sub>4</sub> .....	0.7g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.7g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
NaCl.....	5.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0mg
ZnSO <sub>4</sub> .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	1.0mg

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For determination of the susceptibility of plastics to fungal degradation.

### ASW Medium (Artificial Seawater Medium)

**Composition** per liter:

NaCl.....	27.0g
Agar.....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.5g
KNO <sub>3</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.07g
NaHCO <sub>3</sub> .....	0.04g
Tris-HCl buffer (1.0M, pH 7.6).....	20.0mL
Chelated iron solution.....	1.0mL
Trace metal solution.....	1.0mL

**Trace Metal Solution:**

**Composition** per 100.0mL:

H <sub>3</sub> BO <sub>3</sub> .....	60.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	40.0mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	37.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	4.0mg
ZnCl <sub>2</sub> .....	4.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.5mg

**Preparation of Trace Metal Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Chelated Iron Solution:**

**Composition** per 100.0mL:

FeCl <sub>3</sub> ·4H <sub>2</sub> O.....	240.0mg
EDTA.....	14.6g

**Preparation of Chelated Iron Solution:** Add EDTA to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.6. Add FeCl<sub>3</sub>·4H<sub>2</sub>O. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Porphyridium purpureum*.

### Atlas Oil Agar

**Composition** per liter:

Bushnell-Haas agar.....	990.0mL
Oil.....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Bushnell-Haas Agar:**

**Composition** per 990.0mL:

Agar.....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
FeCl <sub>3</sub> .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g

**Preparation of Bushnell-Haas Agar:** Add components to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C.

**Preparation of Medium:** Filter sterilize oil. Aseptically add 10.0mL of sterile oil to 990.0mL of cooled, sterile Bushnell-Haas agar. Put mixture into a sterile blender container. Blend on low speed to minimize the incorporation of air into the medium. Pour into sterile Petri dishes.

**Use:** For the cultivation and enumeration of hydrocarbon-utilizing bacteria by direct plating of water and sediment samples.

### AT5N Medium

**Composition** per liter:

CaCO <sub>3</sub> .....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> .....	50.0mg
KHCO <sub>3</sub> .....	30.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	20.0mg

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of bacteria that oxidize ammonia, especially those from wastewater.

### Aureomycin® Rose Bengal Glucose Peptone Agar

**Composition** per liter:

Agar.....	20.0g
Glucose.....	10.0g
Peptone.....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Rose Bengal.....	0.035g
Aureomycin solution.....	200.0mL

pH 5.4 ± 0.2 at 25°C

**Aureomycin Solution:**

**Composition** per 200.0mL:

Aureomycin-HCl.....	0.07g
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**Preparation of Aureomycin Solution:** Add aureomycin-HCl to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except aureomycin solution, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 200.0mL of sterile aureomycin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and enumeration of fungi isolated from sewage and polluted waters.

### Autotrophic *Nitrobacter* Medium (DSMZ Medium 756c)

#### Composition per liter:

NaNO <sub>2</sub> .....	2.0g
Stock solution.....	100.0mL
Trace elements solution.....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Stock Solution:

##### Composition per liter:

NaCl.....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

##### Composition per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	97.3mg
H <sub>3</sub> BO <sub>3</sub> .....	49.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	43.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	37.1mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	33.8mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	25.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.6. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Allow to stand for 2–3 days so that pH adjusts itself to 7.4–7.6.

**Use:** For the cultivation of *Nitrobacter winogradskyi*.

### Autotrophic *Nitrobacter* Medium (LMG Medium 247)

#### Composition per liter:

NaNO <sub>2</sub> .....	2.0g
Stock solution.....	100.0mL
Trace elements solution.....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Stock Solution:

##### Composition per liter:

NaCl.....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

##### Composition per liter:

(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .....	437.10mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	97.30mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	43.10mg
H <sub>3</sub> BO <sub>3</sub> .....	39.40mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	33.80mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	25.00mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Adjust pH to 8.6 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Allow the medium to stand for 2–3 days so that the pH can adjust itself to pH 7.4–7.6.

**Use:** For the cultivation of autotrophic *Nitrobacter* spp.

### Azide Blood Agar

#### Composition per liter:

Agar.....	15.0g
Pancreatic digest of casein.....	5.0g
Peptic digest of animal tissue.....	5.0g
NaCl.....	5.0g
Beef extract.....	3.0g
NaN <sub>3</sub> .....	0.2g
Sheep blood, defibrinated.....	50.0mL

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems, and Oxoid Unipath.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components, except sheep blood, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45–50°C. Aseptically add 50.0mL of sterile defibrinated sheep blood. Pour into sterile Petri dishes or distribute into sterile tubes. Allow tubes to cool in a slanted position.

**Use:** For the isolation and differentiation of streptococci and staphylococci from specimens containing mixed flora and from nonclinical specimens such as water and sewage.

### Azide Blood Agar with Crystal Violet (Packer's Agar)

#### Composition per liter:

Agar.....	15.0g
Pancreatic digest of casein.....	5.0g
Peptic digest of animal tissue.....	5.0g
NaCl.....	5.0g
Beef extract.....	3.0g
NaN <sub>3</sub> .....	0.9g
Crystal Violet.....	2.0mg
Sheep blood, defibrinated.....	50.0mL

pH 7.2 ± 0.2 at 25°C

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components, except sheep blood, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 50.0mL of sterile defibrinated sheep blood. Pour into sterile Petri dishes or distribute into sterile tubes. Allow tubes to cool in a slanted position.

**Use:** For the isolation and enumeration of fecal streptococci from nonclinical specimens such as water. Also used for the isolation of *Streptococcus pneumoniae* and *Erysipelothrix rhusiopathiae*.

### Azide Broth (Azide Glucose Broth) (Azide Dextrose Broth)

#### Composition per liter:

Pancreatic digest of casein.....	15.0g
Glucose.....	7.5g
NaCl.....	7.5g



Beef extract .....	4.5g
NaN <sub>3</sub> .....	0.2g
pH 7.2 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Prepare double-strength broth for samples larger than 1.0mL.

**Use:** For the detection and enrichment of fecal streptococci in water and sewage. Also used in the multiple-tube technique as a presumptive test for the presence of fecal streptococci.

**Azide Broth, Rothe**  
(Azide Glucose Broth, Rothe)  
(Azide Dextrose Broth, Rothe)

**Composition per liter:**

Peptone .....	20.0g
Glucose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.7g
KH <sub>2</sub> PO <sub>4</sub> .....	2.7g
NaN <sub>3</sub> .....	0.2g
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Prepare double-strength broth for samples larger than 1.0mL.

**Use:** For the detection of enterococci in water and sewage.

**Azide Citrate Broth**

**Composition per liter:**

Pancreatic digest of casein .....	20.0g
Sodium citrate .....	10.0g
Yeast extract .....	5.0g
Glucose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NaN <sub>3</sub> .....	0.25g
pH 7.0 ± 0.2 at 25°C	

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–118°C. Prepare double-strength broth for samples larger than 1.0mL.

**Use:** For the detection and enrichment of fecal streptococci in water and sewage.

**Azoarcus Medium**  
(LMG Medium 202)

**Composition per liter:**

Solution A .....	750.0mL
Phosphate buffer solution .....	250.0mL
pH 6.8 ± 0.2 at 25°C	

**Solution A:**

**Composition per 750.0mL:**

Malic acid .....	5.0g
KOH .....	4.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g
CaCl <sub>2</sub> .....	20.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	10.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
Ferric EDTA solution .....	10.0mL

**Ferric EDTA Solution:**

**Composition per liter:**

Ferric EDTA .....	0.066g
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**Preparation of Ferric EDTA Solution:** Add ferric EDTA to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Solution A:** Add 5.0g malic acid to 500.0mL distilled/deionized water. Adjust pH to 7.0 with KOH (approximate amount of 4.5g). Add other components. Mix thoroughly. Bring volume to 750.0mL. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Phosphate Buffer Solution:**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	5.8g
KH <sub>2</sub> PO <sub>4</sub> .....	4.5g

**Preparation of Phosphate Buffer Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Aseptically combine 750.0mL sterile solution A with 250.0mL sterile phosphate buffer solution. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Azoarcus indigenus*.

**Azoarcus VM Medium**  
(LMG Medium 252)

**Composition per liter:**

Agar .....	15.0g
Beef extract .....	3.0g
DL-malic acid .....	2.5g
KOH .....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NaCl .....	1.1g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.2g
Fe EDTA .....	66.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	10.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
Biotin .....	0.1mg
NH <sub>4</sub> Cl .....	0.5mg
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Gently heat and bring to boiling. Distribute

ute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azospira oryzae* and *Azonexus fungiphilus*.

#### ***Azorhizobium caulinodans* Agar (LMG 119)**

**Composition per liter:**

Agar .....	15.0g
Beef extract .....	5.0g
Peptone.....	5.0g
Sucrose.....	5.0g
Yeast extract.....	1.0g
MgSO <sub>4</sub> .....	0.24g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azorhizobium caulinodans*.

#### ***Azorhizophilus paspali* Agar**

**Composition per liter:**

Agar .....	20.0g
Sucrose.....	20.0g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
FeCl <sub>3</sub> .....	0.01g

pH 6.9 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azorhizophilus paspali*.

#### ***Azospirillum amazonense* Medium (LGI Medium)**

**Composition per liter:**

Sucrose.....	5.0g
Agar .....	1.75g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g
FeCl <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	2.0mg
Bromothymol Blue solution.....	5.0mL

pH 6.0 ± 0.2 at 25°C

**Bromothymol Blue Solution:**

**Composition per 100.0mL:**

Bromothymol Blue .....	0.5g
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**Preparation of Bromothymol Blue Solution:** Add Bromothymol Blue to 100.0mL of 0.2N KOH. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azospirillum amazonense*.

#### ***Azospirillum lipoferum* Agar Medium**

**Composition per liter:**

Glucose.....	20.0g
Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.8g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.1g
Yeast extract .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.02g

pH 6.9 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Azospirillum lipoferum*.

#### ***Azospirillum lipoferum* Medium**

**Composition per liter:**

Calcium malate.....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Azospirillum lipoferum*.

#### ***Azospirillum* Medium**

**Composition per liter:**

Sodium malate.....	5.0g
Agar .....	1.75g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g
FeCl <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	2.0mg
Bromothymol Blue solution .....	5.0mL

pH 6.8 ± 0.2 at 25°C

**Bromothymol Blue Solution:**

**Composition per 10.0mL:**

Bromothymol Blue .....	0.5g
Ethanol .....	10.0mL

**Preparation of Bromothymol Blue Solution:** Add Bromothymol Blue to 10.0mL of ethanol. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Azospirillum* species isolated from roots.

#### ***Azotobacter* Agar**

**Composition per liter:**

Agar .....	15.0g
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Sucrose.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.002g
FeCl <sub>3</sub> .....	1.0μg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azorhizophilus paspali*.

#### *Azotobacter* Agar, Modified I

##### Composition per liter:

Agar.....	15.0g
Sucrose.....	10.0g
Glucose.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> .....	2.0mg
FeCl <sub>3</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azotobacter* species.

#### *Azotobacter* Agar, Modified II

##### Composition per liter:

Sucrose.....	20.0g
Agar.....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> .....	2.0mg
FeCl <sub>3</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0mg

pH 6.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azotobacter* species and *Beijerinckia dextrii*.

#### *Azotobacter* Basal Agar

##### Composition per liter:

Agar.....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
NaCl.....	0.2g

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0mg
Soil extract.....	100.0mL

pH 7.2 ± 0.2 at 25°C

##### Soil Extract:

##### Composition per 200.0mL:

African Violet soil.....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 200.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

**Preparation of Medium:** Add components, including filtered soil extract, to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of bacteria, including *Azomonas* species, *Azotobacter* species, and others when a carbon source is added.

#### *Azotobacter* Broth

##### Composition per liter:

Glucose.....	10.0g
CaCO <sub>3</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	5.0mg

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azotobacter beijerinckii*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, and *Dexia gummosa*.

#### *Azotobacter* Broth, Modified I

##### Composition per liter:

Sucrose.....	10.0g
Glucose.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> .....	2.0mg
FeCl <sub>3</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Azotobacter* species.

#### *Azotobacter* Broth, Modified II

##### Composition per liter:

Sucrose.....	20.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> .....	2.0mg
FeCl <sub>3</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0mg
pH 6.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Azotobacter* species and *Beijerinckia derxii*.

#### *Azotobacter chroococcum* Agar

**Composition per liter:**

Agar .....	20.0g
CaCO <sub>3</sub> .....	20.0g
Glucose .....	20.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.8g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05g
pH 7.4–7.6 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azotobacter chroococcum*.

#### *Azotobacter* Medium

**Composition per liter:**

Agar .....	15.0g
CaCO <sub>3</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	5.0mg
Glucose solution .....	25.0mL
Mannitol solution .....	25.0mL
pH 7.3 ± 0.2 at 25°C	

#### Glucose Solution:

**Composition per 25.0mL:**

D-Glucose .....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize. Warm to 50°–55°C.

#### Mannitol Solution:

**Composition per 25.0mL:**

Mannitol .....	5.0g
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**Preparation of Mannitol Solution:** Add mannitol to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize. Warm to 50°–55°C.

**Preparation of Medium:** Add components, except glucose solution and mannitol solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 25.0mL of sterile glucose solution

and 25.0mL of sterile mannitol solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Azotobacter* species.

#### *Azotobacter* Medium (ATCC Medium 14)

**Composition per liter:**

Sucrose .....	20.0g
Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.8g
Yeast extract .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1g
FeCl <sub>3</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0mg
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of bacteria, including *Azomonas* species, *Azotobacter* species, *Beijerinckia derxii*, *Pseudomonas azotocolligans*, and *Rhodococcus erythropolis*.

#### *Azotobacter* Medium (ATCC Medium 240)

**Composition per liter:**

Agar .....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
FeCl <sub>3</sub> .....	1.0mg
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour agar medium into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a variety of bacteria, including *Azotobacter* species.

#### *Azotobacter* Medium (ATCC Medium 1771)

**Composition per liter:**

Agar .....	15.0g
Glucose .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.22g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.098g
NaCl .....	0.058g
K <sub>2</sub> HPO <sub>4</sub> .....	0.058g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.2mg
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a variety of bacteria, including *Azotobacter* species.

#### *Azotobacter paspali* Medium

##### Composition per liter:

Agar .....	20.0g
Sucrose .....	20.0g
CaCO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
CaCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
Bromothymol Blue solution .....	10.0mL
FeCl <sub>3</sub> (10% solution) .....	0.1mL
pH 7.0 ± 0.2 at 25°C	

##### Bromothymol Blue Solution:

##### Composition per 10.0mL:

Bromothymol Blue .....	0.5g
Ethanol .....	10.0mL

**Preparation of Bromothymol Blue Solution:** Add Bromothymol Blue to 10.0mL of ethanol. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azotobacter paspali*.

#### *Azotobacter* Supplement (ATCC Medium 11)

##### Composition per liter:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Soil extract .....	100.0mL
Glucose solution .....	100.0mL
pH 7.6 ± 0.2 at 25°C	

##### Soil Extract:

##### Composition per 200.0mL:

African Violet soil .....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 200.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

##### Glucose Solution:

##### Composition per 100.0mL:

Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to tap water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 7.6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Azomonas agilis* and *Azotobacter chroococcum*.

#### *Azotobacter* Supplement (ATCC Medium 12)

##### Composition per liter:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Soil extract .....	100.0mL
Mannitol solution .....	100.0mL
pH 7.6 ± 0.2 at 25°C	

##### Soil Extract:

##### Composition per 200.0mL:

African Violet soil .....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g

**Preparation of Soil Extract:** Add components to distilled/deionized water and bring volume to 200.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

##### Mannitol Solution:

##### Composition per 100.0mL:

Mannitol .....	20.0g
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**Preparation of Mannitol Solution:** Add mannitol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except mannitol solution, to tap water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 7.6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile mannitol solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Azotobacter* species and *Azomonas* species.

#### *Azotobacter* Supplement (ATCC Medium 13)

##### Composition per liter:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Soil extract .....	100.0mL
Glucose solution .....	100.0mL
pH 6.0 ± 0.2 at 25°C	

##### Soil Extract:

##### Composition per 200.0mL:

African Violet soil .....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 200.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

##### Glucose Solution:

##### Composition per 100.0mL:

Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to tap water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 6.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add

sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Beijerinckia* species.

### ***Azotobacter* Supplement (ATCC Medium 15)**

**Composition** per liter:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Soil extract .....	100.0mL
Mannitol solution .....	100.0mL

pH 6.0 ± 0.2 at 25°C

**Soil Extract:**

**Composition** per 200.0mL:

African Violet soil .....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g

**Preparation of Soil Extract:** Add components to distilled/deionized water and bring volume to 200.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

**Mannitol Solution:**

**Composition** per 100.0mL:

Mannitol .....	20.0g
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**Preparation of Mannitol Solution:** Add mannitol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except mannitol solution, to tap water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 6.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile mannitol solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Azomonas macrocytogenes*.

### ***Azotobacter vinelandii* Medium**

**Composition** per liter:

Sodium benzoate .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Mannitol .....	0.5g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Azotobacter vinelandii* from water samples.

### **BA Medium with Cellobiose**

**Composition** per liter:

NaHCO <sub>3</sub> .....	2.6g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	0.75g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Resazurin .....	0.5mg
Cellobiose solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL

pH 6.9–7.0 at 25°C

**Cellobiose Solution:**

**Composition** per 50.0mL:

Cellobiose .....	4.0g
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**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Wolfe's Mineral Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Wolfe's Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except cellobiose solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Wolfe's mineral solution, and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile cellobiose solution, 10.0mL of sterile Wolfe's mineral solution, 10.0mL of sterile Wolfe's vitamin solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Caldicellulosiruptor lactoaceticus*.

**Baar's Medium for Sulfate Reducers****Composition** per liter:

Sodium lactate .....	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
CaSO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
Ferrous ammonium sulfate solution .....	10.0mL
Yeast extract solution .....	10.0mL
pH 7.5 ± 0.2 at 25°C	

**Ferrous Ammonium Sulfate Solution:****Composition** per 10.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	0.5g
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**Preparation of Ferrous Ammonium Sulfate Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Yeast Extract Solution:****Composition** per 10.0mL:

Yeast extract .....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except ferrous ammonium sulfate solution and yeast extract solution, to tap water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile ferrous ammonium sulfate solution and sterile yeast extract solution. Mix thoroughly. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfotomaculum nigrificans*.

**Baar's Medium  
for Sulfate Reducers, Modified****Composition** per 1020.0mL:

Component I .....	400.0mL
Component III .....	400.0mL
Component II .....	200.0mL
Ferrous ammonium sulfate solution .....	20.0mL
pH 7.5 ± 0.2 at 25°C	

**Component I:****Composition** per 400.0mL:

Sodium citrate .....	5.0g
MgSO <sub>4</sub> .....	2.0g
CaSO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g

**Preparation of Component I:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Component II:****Composition** per 200.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
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**Preparation of Component II:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Component III:****Composition** per 400.0mL:

Sodium lactate .....	3.5g
Yeast extract .....	1.0g

**Preparation of Component III:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Ferrous Ammonium Sulfate Solution:****Composition** per 20.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	1.0g
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**Preparation of Ferrous Ammonium Sulfate Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine component I, component II, and component III. Mix thoroughly. Distribute 5.0mL volumes into tubes under 97% N<sub>2</sub> + 3% H<sub>2</sub>. Add medium to tubes while still warm to exclude as much O<sub>2</sub> as possible. Aseptically add 0.1mL of sterile ferrous ammonium sulfate solution to 5.0mL of medium immediately prior to inoculation.

**Use:** For the cultivation and maintenance of *Desulfovibrio*, *Desulfobulbus*, *Desulfotomaculum*, and *Thermodesulfobacterium* species.

**Baar's Medium for Sulfate Reducers, Modified  
with 2.5% NaCl****Composition** per 1020.0mL:

Component I .....	400.0mL
Component III .....	400.0mL
Component II .....	200.0mL
Ferrous ammonium sulfate solution .....	20.0mL
pH 7.5 ± 0.2 at 25°C	

**Component I:****Composition** per 400.0mL:

NaCl .....	25.0g
Sodium citrate .....	5.0g
MgSO <sub>4</sub> .....	2.0g
CaSO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g

**Preparation of Component I:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Component II:****Composition** per 200.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
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**Preparation of Component II:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Component III:****Composition** per 400.0mL:

Sodium lactate .....	3.5g
Yeast extract .....	1.0g

**Preparation of Component III:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Ferrous Ammonium Sulfate Solution:****Composition** per 20.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	1.0g
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**Preparation of Ferrous Ammonium Sulfate Solution:** Add  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$  to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine component I, component II, and component III. Mix thoroughly. Distribute 5.0mL volumes into tubes under 97%  $\text{N}_2$  + 3%  $\text{H}_2$ . Add medium to tubes while still warm to exclude as much  $\text{O}_2$  as possible. Aseptically add 0.1mL of sterile ferrous ammonium sulfate solution to 5.0mL of medium immediately prior to inoculation.

**Use:** For the cultivation of *Desulfovibrio africanus* and other *Desulfovibrio* species that prefer 2.5% NaCl.

#### *Bacillus acidocaldarius* Agar

**Composition** per liter:

Solution A .....	500.0mL
Solution B .....	500.0mL
pH 3.0–4.0 at 25°C	

#### **Solution A:**

**Composition** per 500.0mL:

$\text{KH}_2\text{PO}_4$ .....	3.0g
Yeast extract .....	1.0g
Glucose .....	1.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.25g
$(\text{NH}_4)_2\text{SO}_4$ .....	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Adjust pH to 3.0–4.0. Mix thoroughly. Autoclave for 10 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### **Solution B:**

**Composition** per 500.0mL:

Agar .....	30.0g
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**Preparation of Solution B:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically mix 500.0mL of solution A and 500.0mL of solution B. Mix thoroughly. Aseptically adjust pH to 3.0–4.0. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus (Alicyclobacillus) acidocaldarius*.

#### *Bacillus acidoterrestris* Agar

**Composition** per 1001.0mL:

Solution A .....	500.0mL
Solution C .....	500.0mL
Solution B (Trace elements solution SL-6) .....	1.0mL
pH 4.0 ± 0.2 at 25°C	

#### **Solution A:**

**Composition** per 500.0mL:

Glucose .....	5.0g
$\text{KH}_2\text{PO}_4$ .....	3.0g
Yeast extract .....	2.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.25g
$(\text{NH}_4)_2\text{SO}_4$ .....	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

**Composition** per 500.0mL:

Agar .....	15.0g
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**Preparation of Solution C:** Add agar to distilled/deionized water and bring volume to 500.0mL. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### **Solution B (Trace Elements Solution SL-6):**

**Composition** per liter:

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	0.5g
$\text{H}_3\text{BO}_3$ .....	0.3g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.2g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	0.03g
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.02g
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.01g

**Preparation of Solution B (Trace Elements Solution SL-6):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 500.0mL of sterile solution A, 500.0mL of sterile solution C, and 1.0mL of sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus acidoterrestris*, *Alicyclobacillus acidoterrestris*, and *Alicyclobacillus cycloheptanicus*.

#### *Bacillus* Agar

**Composition** per liter:

Agar .....	20.0g
$(\text{NH}_4)_2\text{SO}_4$ .....	1.3g
Glucose .....	1.0g
Yeast extract .....	1.0g
$\text{KH}_2\text{PO}_4$ .....	0.37g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.25g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.07g
$\text{FeCl}_3$ .....	0.02g

pH 4.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 3.5. Prepare a separate agar solution by adding 20.0g/500.0mL of distilled/deionized water. Autoclave solutions separately for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically combine both solutions. This procedure avoids acid hydrolysis of the agar. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of acidophilic *Bacillus* species such as *Bacillus acidocaldarius*.

#### *Bacillus* Agar, Modified

**Composition** per liter:

Agar .....	20.0g
Glucose .....	1.0g
Yeast extract .....	1.0g
$\text{KH}_2\text{PO}_4$ .....	0.6g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.25g
$(\text{NH}_4)_2\text{SO}_4$ .....	0.2g

pH 3.0–4.0 at 25°C

**Preparation of Medium:** Add components, except agar and glucose, to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boil-



ing. Adjust pH to 3.5. Prepare a separate agar and glucose solution by adding 20.0g of agar and 1.0g of glucose to 500.0mL of distilled/deionized water. Autoclave solutions separately for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically combine both solutions. This procedure avoids acid hydrolysis of the agar. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of acidophilic *Bacillus* species such as *Bacillus acidocaldarius*.

#### *Bacillus benzoovorans* Agar

##### Composition per liter:

Agar .....	15.0g
Yeast extract .....	6.0g
Peptone .....	5.0g
NaCl .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	3.6g
Sodium benzoate .....	2.0g
Beef extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.98g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.03g
Trace elements solution .....	0.2mL
pH 7.0–7.2 at 25°C	

##### Trace Elements Solution:

##### Composition per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus benzoovorans*.

#### *Bacillus* Broth

##### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Glucose .....	1.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.37g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeCl <sub>3</sub> .....	0.02g
pH 4.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 4.0 with 10N H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of acidophilic *Bacillus* species such as *Bacillus acidocaldarius*.

#### *Bacillus cycloheptanicus* Agar

##### Composition per 1001.0mL:

Solution A .....	500.0mL
Solution C .....	500.0mL
Solution B (Trace Elements solution SL-6) .....	1.0mL
pH 4.0 ± 0.2 at 25°C	

#### Solution A:

##### Composition per liter:

Yeast extract .....	5.0g
Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

##### Composition per 500.0mL:

Agar .....	15.0g
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**Preparation of Solution C:** Add agar to distilled/deionized water and bring volume to 500.0mL. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Solution B (Trace Elements Solution SL-6):

##### Composition per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Solution B (Trace Elements Solution SL-6):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 500.0mL of sterile solution A, 500.0mL of sterile solution C, and 1.0mL of sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus cycloheptanicus*, *Alicyclobacillus acidoterrestris*, and *Alicyclobacillus cycloheptanicus*.

#### *Bacillus halodenitrificans* Agar (LMG Medium 142)

##### Composition per liter:

NaCl .....	100.0g
Agar .....	15.0g
Sodium acetate·3H <sub>2</sub> O .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	3.8g
KH <sub>2</sub> PO <sub>4</sub> .....	1.3g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Mg(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
Yeast extract .....	1.0g
Magnesium nitrate solution .....	100.0mL
pH 7.2 ± 0.2 at 25°C	

##### Magnesium Nitrate Solution:

##### Composition per 100.0mL:

Mg(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
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**Preparation of Magnesium Nitrate Solution:** Add Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except magnesium nitrate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 7.2 with KOH. Autoclave for 15 min at 15 psi pressure–121°C.

Cool to 45°–50°C. Aseptically add 100.0mL sterile magnesium nitrate solution. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Bacillus halodentrificans*.

#### ***Bacillus* Medium (ATCC Medium 21)**

**Composition** per liter:

Glycerol .....	20.0g
L-Glutamic acid.....	4.0g
Citric acid.....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Ferric ammonium citrate.....	0.5g
MgSO <sub>4</sub> .....	0.5g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus licheniformis*.

#### ***Bacillus* Medium (ATCC Medium 455)**

**Composition** per liter:

Soluble starch.....	30.0g
Agar .....	20.0g
Polypeptone™.....	5.0g
Yeast extract.....	5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Swirl medium to resuspend starch. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus subtilis*. Also used to detect amylase-producing microorganisms.

#### ***Bacillus pasteurii* Agar**

**Composition** per liter:

Urea.....	20.0g
Agar .....	15.0g
Peptone.....	5.0g
Meat extract .....	3.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus pasteurii* and *Sporosarcina ureae*.

#### ***Bacillus polymyxa* Agar**

**Composition** per liter:

Agar .....	20.0g
Starch, soluble.....	10.0g
Peptone.....	5.0g
Yeast extract.....	5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus macerans*, *Bacillus polymyxa*, and *Bacillus thermoglucosidarius*.

#### ***Bacillus popilliae* Medium**

**Composition** per liter:

Yeast extract .....	10.0g
Acid hydrolysate of casein.....	7.95g
K <sub>2</sub> HPO <sub>4</sub> .....	3.0g
Beef extract .....	1.36g
Trehalose .....	1.0g
Starch.....	0.68g

pH 7.3 ± 0.1 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring to 1.0L. Mix thoroughly. Gently heat until dissolved. Do not overheat. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Bacillus popilliae*.

#### ***Bacillus* Pullulan Salts**

**Composition** per liter:

Pullulan.....	2.5g
NaCl .....	1.0g
NH <sub>4</sub> Cl.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Yeast extract .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace mineral solution.....	10.0mL
Vitamin solution .....	10.0mL

pH 6.0 ± 0.2 at 25°C

#### **Trace Mineral Solution:**

**Composition** per liter:

CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.13g
ZnCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	20.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	20.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	20.0mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0mg
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.4mg
KI.....	0.1mg

**Preparation of Trace Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate.....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Thioctic acid.....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Bacillus* species that can degrade pullulan.

***Bacillus schlegelii* Agar  
(LMG Medium 85)**

**Composition** per liter:

Agar .....	30.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12 H <sub>2</sub> O .....	9.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
Sodium pyruvate .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	10.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Ferric ammonium citrate .....	5.0mg
Trace elements solution .....	3.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Bacillus schlegelii*.

***Bacillus schlegelii*  
Chemolithotrophic Growth Medium  
(DSMZ Medium 261)**

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	4.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate .....	5.0mg
Trace elements solution SL-6 .....	3.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Elements Solution SL-6:**

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
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**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the chemolithotrophic cultivation of *Bacillus schlegelii*. Incubation is at 65°C under an atmosphere of 5% O<sub>2</sub>, 10% CO<sub>2</sub>, 45% H<sub>2</sub>.

***Bacillus schlegelii*  
Heterotrophic Growth Medium  
(DSMZ Medium 260)**

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	4.5g
Na-Pyruvate .....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate .....	5.0mg
Trace elements solution SL-6 .....	3.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Elements Solution SL-6:**

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the heterotrophic cultivation of *Bacillus schlegelii*. Incubation is at 65°C.

***Bacillus selenitireducens* Medium  
(DSMZ Medium 968)**

**Composition** per liter:

NaCl .....	90.0g
Na <sub>2</sub> CO <sub>3</sub> .....	10.6g
NaHCO <sub>3</sub> .....	4.2g
Na-lactate .....	1.70g
NaNO <sub>3</sub> .....	1.25g
Yeast extract .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.15g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	0.08g
MgSO <sub>4</sub> .....	25.0mg
Resazurin .....	0.5mg
Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
Selenite-tungstate solution .....	1.0mL

pH 9.8 ± 0.2 at 25°C

# Cysteine Solution:

## Composition per 10.0mL:

L-Cysteine-HCl-H<sub>2</sub>O ..... 0.25g

**Preparation of Cysteine Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

# Na<sub>2</sub>S-9H<sub>2</sub>O Solution:

## Composition per 10.0mL:

Na<sub>2</sub>S-9H<sub>2</sub>O ..... 0.25g

**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 25°C.

# Trace Elements Solution SL-10:

## Composition per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

## Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

# Selenite-Tungstate Solution:

## Composition per liter:

NaOH ..... 0.5g  
Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O ..... 4.0mg  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>, cysteine solution, and Na<sub>2</sub>S-9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> gas. Add solid NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>. Adjust pH to 9.8. Distribute to anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure-121°C. Cool to room temperature. Aseptically and anaerobically add per liter 10.0mL sterile cysteine solution and 10.0mL sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Bacillus selenitireducens* and *Bacillus arseniciselenatis*.

## *Bacillus stearothermophilus* Broth

## Composition per liter:

Pancreatic digest of casein ..... 10.0g  
Yeast extract ..... 5.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 2.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Bacillus stearothermophilus*.

## *Bacillus stearothermophilus* Defined Broth

## Composition per 100.0mL:

Mineral salts solution ..... 10.0mL  
Potassium phosphate buffer ..... 5.0mL  
L-Glutamate-HCl (1% solution) ..... 4.0mL  
L-Leucine (1% solution) ..... 1.64mL  
L-Lysine-HCl (1% solution) ..... 1.4mL  
L-Serine (1% solution) ..... 1.4mL  
L-Aspartate (1% solution) ..... 1.3mL  
L-Valine (1% solution) ..... 1.26mL  
Biotin (0.01% solution) ..... 1.0mL  
Glucose (20% solution) ..... 1.0mL  
L-Isoleucine (1% solution) ..... 1.0mL  
L-Proline (1% solution) ..... 1.0mL  
Nicotinic acid (0.01% solution) ..... 1.0mL  
Thiamine-HCl (0.01% solution) ..... 1.0mL  
L-Phenylalanine (1% solution) ..... 0.86mL  
L-Alanine (1% solution) ..... 0.84mL  
L-Threonine (1% solution) ..... 0.84mL  
L-Arginine-HCl (1% solution) ..... 0.64mL  
L-Tyrosine (1% solution) ..... 0.56mL  
L-Methionine (1% solution) ..... 0.52mL  
Glycine (1% solution) ..... 0.5mL  
L-Asparagine-H<sub>2</sub>O (1% solution) ..... 0.5mL  
L-Cystine (1% solution) ..... 0.5mL  
L-Glutamine (1% solution) ..... 0.5mL  
L-Histidine-HCl-H<sub>2</sub>O (1% solution) ..... 0.42mL  
L-Tryptophan (1% solution) ..... 0.3mL  
CaCl<sub>2</sub> (5% solution) ..... 0.01mL  
FeCl<sub>3</sub>·6H<sub>2</sub>O (0.05% solution) ..... 0.01mL  
MnCl<sub>2</sub> (10mm solution) ..... 0.01mL  
ZnSO<sub>4</sub>·7H<sub>2</sub>O (5% solution) ..... 0.01mL

pH 7.3 ± 0.2 at 25°C

## Mineral Salts Solution:

## Composition per liter:

NaCl ..... 10.0g  
NH<sub>4</sub>Cl ..... 10.0g  
MgSO<sub>4</sub> ..... 4.0g

**Preparation of Mineral Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

## Potassium Phosphate Buffer:

## Composition per 500.0mL:

K<sub>2</sub>HPO<sub>4</sub> ..... 125.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 30.0g

**Preparation of Potassium Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Use:** For the cultivation of *Bacillus stearothermophilus* in a chemically defined medium.

## *Bacillus thermoalcalophilus* Medium

## Composition per liter:

Yeast extract ..... 10.0g  
Sodium acetate ..... 3.0g  
KCl ..... 1.8g  
Na<sub>2</sub>SO<sub>4</sub> ..... 0.4g

K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> .....	0.2g
FeSO <sub>4</sub> .....	0.01g

pH 8.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus thermoalcalophilus*.

#### *Bacillus thermoantarcticus* Medium

**Composition** per liter:

Yeast extract.....	6.0g
NaCl.....	3.0g
Soil extract.....	500.0mL

pH 5.6–5.8 at 25°C

**Soil Extract:**

**Composition** per liter:

Garden soil, air dried .....	400.0g
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**Preparation of Soil Extract:** Pass 400.0g of air-dried garden soil through a coarse sieve. Add soil to 960.0mL of tap water. Mix thoroughly. Autoclave for 60 min at 15 psi pressure–121°C. Cool to room temperature. Allow residue to settle. Decant supernatant solution. Filter through Whatman filter paper. Distribute into bottles in 200.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature until clear.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.6–5.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus thermoantarcticus*.

#### *Bacillus thermoglucosidasius* Agar

**Composition** per liter:

Agar .....	30.0g
Soluble starch.....	10.0g
Peptone.....	5.0g
Beef extract.....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract.....	3.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus thermoglucosidasius*.

#### *Bacillus thermoleovorans* Medium

**Composition** per liter:

<i>n</i> -Heptadecane .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Yeast extract.....	1.0g
KCl.....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus thermoleovorans*.

#### *Bacillus thuringiensis* Medium

**Composition** per liter:

Glucose.....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Yeast extract .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.08g
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	0.05g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus thuringiensis*.

#### *Bacillus tusciae* Medium

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.3g
NH <sub>4</sub> Cl.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
NaHCO <sub>3</sub> .....	0.5g
Fe(NH <sub>4</sub> ) citrate .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.01g
Ferric ammonium citrate solution .....	20.0mL
Trace elements solution SL-6.....	5.0mL

pH 4.0 ± 0.2 at 25°C

**Ferric Ammonium Citrate Solution:**

**Composition** per 20.0mL:

Ferric ammonium citrate .....	0.05g
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**Preparation of Ferric Ammonium Citrate Solution:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-6:**

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except ferric ammonium citrate solution and trace elements solution SL-6, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile ferric ammonium citrate solution and 5.0mL of sterile trace elements solution SL-6. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. For chemolithotrophic growth, incubate the culture under 2% O<sub>2</sub> + 10% CO<sub>2</sub> + 60% H<sub>2</sub> + 28% N<sub>2</sub>.

**Use:** For the chemolithotrophic growth of *Bacillus tusciae*.

### *Bacillus tusciae* Medium

#### Composition per liter:

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.3g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaHCO <sub>3</sub> .....	0.5g
Fe(NH <sub>4</sub> ) citrate .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate solution .....	20.0mL
Carbon source .....	10.0mL
Trace elements solution SL-6 .....	5.0mL

pH 4.0 ± 0.2 at 25°C

#### Ferric Ammonium Citrate Solution:

##### Composition per 20.0mL:

Ferric ammonium citrate .....	0.05g
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**Preparation of Ferric Ammonium Citrate Solution:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Carbon Source:

##### Composition per 10.0mL:

Carbohydrate .....	2.0g
or organic acid .....	1.0g

**Preparation of Carbon Source:** Add carbohydrate or organic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution SL-6:

##### Composition per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except ferric ammonium citrate solution, trace elements solution SL-6, and carbon source, to distilled/deionized water and bring volume to 965.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile ferric ammonium citrate solution, 10.0mL of sterile carbon source, and 5.0mL of sterile trace elements solution SL-6. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the heterotrophic growth of *Bacillus tusciae*.

### Bacterial Cell Agar (BCA)

#### Composition per liter:

Tryptose .....	17.36g
Agar .....	15.0g
NaCl .....	8.68g
Beef extract .....	5.2g
Yeast extract .....	1.7g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 30°C. Inoculate with a culture of *Aeromonas hydrophila*. Incubate with shaking at 30°C for 72 hr. Centrifuge culture in 40.0mL volumes at 10,000 × g for 10 min. Wash the cells four times in sterile 0.85% saline. Resuspend the cell pellet in 25.0mL of distilled/deionized water. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. In a separate flask, add 15.0g of agar to 1.0L of distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine 25.0mL of washed cells and 250.0mL of cooled, sterile agar solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the cultivation of freshwater *Myxobacterium* species.

### *Bacteroides cellulosolvens* Medium

#### Composition per liter:

Cellobiose or cellulose .....	5.0g
NaHCO <sub>3</sub> .....	2.0g
NH <sub>4</sub> Cl .....	0.68g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.25g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.18g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5 g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18 g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025 g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3 mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to approximately 500.0mL distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg

Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Cellobiose Solution:**

**Composition per 50.0mL:**

D-Cellobiose (or cellulose) .....	5.0g
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**Preparation of Cellobiose Solution:** Add cellobiose (or cellulose) to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cellobiose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 50.0mL of sterile cellobiose (or cellulose) solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Bacteroides cellulosolvens*.

#### **Baird-Parker Agar**

**Composition per liter:**

Agar .....	17.0g
Glycine.....	12.0g
Sodium pyruvate.....	10.0g
Pancreatic digest of casein.....	10.0g
Beef extract .....	5.0g
LiCl .....	5.0g
Yeast extract.....	1.0g

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath and BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Pour into sterile Petri dishes.

**Use:** Used as a base for the preparation of egg-tellurite-glycine-pyruvate agar for the selective isolation and enumeration of coagulase-positive staphylococci from soil, air, and other materials.

#### **Baird-Parker Agar, Supplemented**

**Composition per liter:**

Agar .....	17.0g
Glycine.....	12.0g
Sodium pyruvate.....	10.0g
Pancreatic digest of casein.....	10.0g
Beef extract .....	5.0g
LiCl .....	5.0g
Yeast extract.....	1.0g
RPF supplement.....	100.0mL

pH 7.0 ± 0.2 at 25°C

#### **RPF Supplement:**

**Composition per 100.0mL:**

Bovine fibrinogen .....	3.75g
Trypsin inhibitor .....	25.0mg
K <sub>2</sub> TeO <sub>3</sub> .....	25.0mg
Rabbit plasma .....	25.0mL

**Caution:** Potassium tellurite is toxic.

**Preparation of RPF Supplement:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except RPF supplement, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of filter-sterilized RPF supplement. Mix thoroughly but gently. Pour into sterile Petri dishes.

**Use:** For the selective isolation and enumeration of coagulase-positive staphylococci from soil, air, and other materials. For the differentiation and identification of staphylococci on the basis of their ability to coagulate plasma. Colonies surrounded by an opaque zone of coagulated plasma are diagnostic for *Staphylococcus aureus*.

#### **BAM Agar (ATCC Medium 1655)**

**Composition per liter:**

Agar .....	30.0g
Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
Trace elements.....	1.0mL

pH 4.0 ± 0.2 at 25°C

#### **Trace Elements:**

**Composition per liter:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.66g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.3g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.18g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.16g
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	0.15g
H <sub>3</sub> BO <sub>3</sub> .....	0.1g

**Preparation of Trace Elements:** Add components to 1.0L of distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust medium to pH 4.0 with H<sub>2</sub>SO<sub>4</sub>. Add agar to 200.0mL of distilled/deionized water. Autoclave agar separately to avoid acid hydrolysis. Autoclave for 15 min at 15 psi pressure–121°C. Mix the two solutions together. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus acidoterrestris*.

#### **BAM SM Agar (ATCC Medium 1656)**

**Composition per liter:**

Agar .....	20.0g
Yeast extract .....	6.0g
Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
Trace elements.....	1.0mL

pH 4.0 ± 0.2 at 25°C

**Trace Elements:****Composition per liter:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.66g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.3g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.18g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.16g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.15g
H <sub>3</sub> BO <sub>3</sub> .....	0.1g

**Preparation of Trace Elements:** Add components to 1.0L of distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust medium to pH 4.0 with H<sub>2</sub>SO<sub>4</sub>. Add agar to 200.0mL of distilled/deionized water. Autoclave agar separately to avoid acid hydrolysis. Autoclave for 15 min at 15 psi pressure–121°C. Mix the two solutions together. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus cycloheptanicus*.

**BAM SM Agar, Modified****Composition per liter:**

Agar .....	30.0g
Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
Trace elements .....	1.0mL

pH 4.0 ± 0.2 at 25°C

**Trace Elements:****Composition per liter:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.66g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.30g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.18g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.16g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.15g
H <sub>3</sub> BO <sub>3</sub> .....	0.10g

**Preparation of Trace Elements:** Add components to 1.0L of distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust medium to pH 4.0 with H<sub>2</sub>SO<sub>4</sub>. Add agar to 200.0mL of distilled/deionized water. Autoclave agar separately to avoid acid hydrolysis. Autoclave for 15 min at 15 psi pressure–121°C. Mix the two solutions together. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus cycloheptanicus*.

**Basal Mineral Medium****Composition per liter:**

NH <sub>4</sub> Cl .....	0.8g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Disodium EDTA .....	9.2mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0mg
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
H <sub>3</sub> BO <sub>3</sub> .....	0.1mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg

MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.02mg
Co(NO <sub>3</sub> ) <sub>2</sub> .....	0.01mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5μg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Use:** For the cultivation of *Beggiatoa* species.

**Basal Thermophile Medium****Composition per liter:**

Solution 1 .....	850.0mL
Solution 2 .....	100.0mL
Solution 3 .....	50.0mL

**Solution 1:****Composition per 850.0mL:**

Pancreatic digest of casein .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Trace elements solution .....	9.0mL
Vitamin solution .....	5.0mL
Resazurin (0.2% solution) .....	1.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (10% solution) .....	0.03mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 850.0mL. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 2:****Composition per 100.0mL:**

Yeast extract .....	3.0g
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**Preparation of Solution 2:** Add yeast extract to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 3:****Composition per 50.0mL:**

Glucose .....	5.0g
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**Preparation of Solution 3:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution:****Composition per liter:**

Nitritotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 100.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components and bring volume to 1.0L. Mix thoroughly.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl .....	10.0mg
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Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine solution 1, solution 2, and solution 3 under 100% N<sub>2</sub>. Distribute into tubes in 10.0mL volumes under 100% N<sub>2</sub>. Immediately prior to inoculation, aseptically add 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube.

**Use:** For the cultivation and maintenance of *Clostridium* species, *Fervidobacterium nodosum*, and *Thermoanaerobium brockii*.

### **BC Medium**

(Medium for *Acetivibrio cellulolyticus*)

**Composition per liter:**

Cellulose powder .....	3.0g
NaHCO <sub>3</sub> .....	2.0g
Mineral solution 1 .....	75.0mL
Mineral solution 2 .....	75.0mL
Cysteine-sulfide reducing solution .....	12.8mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	10.0mL
Vitamin mixture .....	10.0mL
Wolfe's mineral solution .....	10.0mL
Resazurin (0.1% solution) .....	1.0mL

pH 7.6 ± 0.2 at 25°C

**Caution:** This medium contains sodium sulfide and may produce toxic H<sub>2</sub>S gas. Prepare in a chemical fume hood.

#### **Mineral Solution 1:**

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	3.9g
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**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Mineral Solution 2:**

**Composition per liter:**

NH <sub>4</sub> Cl .....	12.0g
Na <sub>2</sub> SO <sub>4</sub> .....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	2.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.8g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:**

**Composition per 100.0mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
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**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Dissolve FeSO<sub>4</sub>·7H<sub>2</sub>O in 100.0mL of distilled/deionized water. Add three drops of concentrated HCl. Mix thoroughly.

#### **Vitamin Mixture:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Cyanocobalamin .....	5.0mg
Lipoic acid (thioctic acid) .....	5.0mg
Biotin .....	2.0mg
<i>p</i> -Aminobenzoic acid .....	0.5mg

**Preparation of Vitamin Mixture:** Add components to distilled/deionized water and bring volume to 1.0L. Store below –20°C.

#### **Wolfe's Mineral Solution:**

**Composition per liter**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriloacetic acid .....	1.5g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
NaCl .....	1.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water and adjust to pH 6.5 with KOH to dissolve. Bring volume to 1.0L with distilled/deionized water. Add remaining components one at a time.

#### **Cysteine-Sulfide Reducing Solution:**

**Composition per 200.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	2.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.5g

**Preparation of Cysteine-Sulfide Reducing Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to 50.0mL of distilled/deionized water. Quickly adjust pH to 10 with fresh 3N NaOH and flush under 100% N<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Bring volume to 200.0mL with distilled/deionized water. Boil under 100% N<sub>2</sub>. Transfer anaerobically to tubes or flasks and stopper. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add cellulose and NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 800.0mL. Add all other components except cysteine-sulfide reducing solution. Heat and boil under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Cool and continue flushing under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. The pH should be 7.6 at room temperature; do not adjust. Add 8.0mL of cysteine-sulfide reducing solution. Add 4.8mL more of cysteine-sulfide reducing solution. Distribute anaerobically into tubes in 7.0mL volumes and cap.

**Use:** For the cultivation and maintenance of *Acetivibrio cellulolyticus*, *Acetivibrio cellulosolvens*, *Bacteroides cellulosolvens*, and other cellulose-degrading microorganisms.

### **BCP Azide Broth**

(Bromcresol Purple Azide Broth)

**Composition per liter:**

Casein peptone .....	10.0g
Yeast extract .....	10.0g

D-Glucose.....	5.0g
NaCl.....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.7g
KH <sub>2</sub> PO <sub>4</sub> .....	2.7g
NaN <sub>3</sub> .....	0.5g
Bromcresol Purple.....	0.032g

pH 6.9 ± 0.2 at 25°C

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components distilled/deionized water to 1.0L. Mix thoroughly. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For use in the confirmation test for the presence of fecal streptococci in water and wastewater.

#### BCYE Agar (BCYE Alpha Base)

##### (Buffered Charcoal Yeast Extract Agar)

**Composition per liter:**

Agar .....	15.0g
Yeast extract.....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid).....	10.0g
Charcoal, activated.....	2.0g
α-Ketoglutarate.....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.4g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O.....	0.25g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except cysteine, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of a 10% solution of L-cysteine-HCl·H<sub>2</sub>O that has been filter sterilized. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental water samples.

#### BCYE Differential Agar (Buffered Charcoal Yeast Extract Differential Agar)

**Composition per liter:**

Agar .....	15.0g
Yeast extract.....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid).....	10.0g
Charcoal, activated.....	2.0g
α-Ketoglutarate.....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.4g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O.....	0.25g
Bromcresol Purple.....	0.01g
Bromothymol Blue.....	0.01g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except L-cysteine-HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9

with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of a 10% solution of L-cysteine-HCl·H<sub>2</sub>O that has been filter sterilized. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental water samples. For the presumptive differential identification of *Legionella* species based on colony color and morphology. *Legionella pneumophila* appears as light blue/green colonies. *Legionella micdadei* appears as blue/gray or dark blue colonies.

#### BCYE Selective Agar with CCVC

##### (Buffered Charcoal Yeast Extract Selective Agar with Cephalothin,

##### Colistin, Vancomycin, and Cycloheximide)

**Composition per 1014.0mL:**

Agar.....	15.0g
Yeast extract.....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid).....	10.0g
Charcoal, activated.....	2.0g
α-Ketoglutarate.....	1.0g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O.....	0.25g
Antibiotic solution.....	10.0mL
Cysteine-HCl·H <sub>2</sub> O solution.....	4.0mL

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

#### L-Cysteine-HCl·H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O.....	1.0g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Antibiotic Solution:

**Composition per 10.0mL:**

Cycloheximide.....	80.0mg
Colistin.....	16.0mg
Cephalothin.....	4.0mg
Vancomycin.....	0.5mg

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine and antibiotic solutions, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of L-cysteine-HCl·H<sub>2</sub>O solution and 10.0mL of sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples. For the selective recovery of *Legionella pneumophila* while reducing contaminating microorganisms from environmental water samples.

**BCYE Selective Agar with GPVA  
(Buffered Charcoal Yeast Extract  
Selective Agar with Glycine, Polymyxin B, Vanco-  
mycin, and Anisomycin)**

**Composition per 1014.0mL:**

Agar .....	15.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	1.0g
$\text{Fe}_4(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$ .....	0.25g
Antibiotic solution .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	4.0mL
pH 6.9 $\pm$ 0.2 at 25°C	

**L-Cysteine-HCl-H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	1.0g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Antibiotic Solution:****Composition per 10.0mL:**

Glycine .....	3.0g
Anisomycin .....	0.08g
Vancomycin .....	5.0mg
Polymyxin B .....	100,000U

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O solution and antibiotic solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of L-cysteine-HCl-H<sub>2</sub>O solution and 10.0mL of sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples. For the selective recovery of *Legionella pneumophila* while reducing contaminating microorganisms from potable water samples.

**BCYE Selective Agar with GVPC  
(Buffered Charcoal Yeast Extract  
Selective Agar with Glycine, Vancomycin, Poly-  
myxin B, and Cycloheximide)**

**Composition per 1014.0mL:**

Agar .....	15.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	1.0g
$\text{Fe}_4(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$ .....	0.25g
Antibiotic solution .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	4.0mL
pH 6.9 $\pm$ 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	1.0g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Antibiotic Solution:****Composition per 10.0mL:**

Glycine .....	3.0g
Cycloheximide .....	0.08g
Vancomycin .....	1.0mg
Polymyxin B .....	79,200U

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O solution and antibiotic solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of L-cysteine-HCl-H<sub>2</sub>O solution and 10.0mL of sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* from environmental samples. For the selective recovery of *Legionella pneumophila* while reducing contaminating microorganisms from potable water samples.

**BCYE Selective Agar with PAC  
(Buffered Charcoal Yeast Extract  
Selective Agar with Polymyxin B,  
Anisomycin, and Cefamandole)**

**Composition per 1014.0mL:**

Agar .....	15.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	1.0g
$\text{Fe}_4(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$ .....	0.25g
Antibiotic solution .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	4.0mL
pH 6.9 $\pm$ 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	1.0g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Antibiotic Solution:****Composition per 10.0mL:**

Polymyxin B .....	80,000 units
Anisomycin .....	80.0mg
Cefamandole .....	2.0mg

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O solution and antibiotic solution, to distilled/deionized water and bring volume to 1.0L. Mix thor-

oughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 50°–55°C. Add 4.0mL of L-cysteine-HCl-H<sub>2</sub>O solution and 10.0mL of sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples. For the selective recovery of *Legionella pneumophila* while reducing contaminating microorganisms from potable water samples.

**BCYE Selective Agar with PAV  
(Buffered Charcoal Yeast Extract  
Selective Agar with Polymyxin B,  
Anisomycin, and Vancomycin)  
(Wadowsky–Yee Medium)**

**Composition per 1014.0mL:**

Agar .....	15.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	1.0g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.25g
Antibiotic solution .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	4.0mL
pH 6.9 $\pm$ 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	1.0g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Antibiotic Solution:**

**Composition per 10.0mL:**

Polymyxin B .....	40,000 units
Anisomycin .....	80.0mg
Vancomycin .....	0.5mg

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine and antibiotic solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 50°–55°C. Add 4.0mL of L-cysteine-HCl-H<sub>2</sub>O solution and 10.0mL of sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples. For the selective recovery of *Legionella pneumophila* while reducing contaminating microorganisms from potable water samples.

**BCYE $\alpha$  with Alb  
(Buffered Charcoal Yeast  
Extract Agar with Albumin)**

**Composition per liter:**

Agar .....	15.0g
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Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	1.0g
Bovine serum albumin solution .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	10.0mL
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O solution .....	10.0mL
pH 6.9 $\pm$ 0.2 at 25°C	

**Bovine Serum Albumin Solution:**

**Composition per 10.0mL:**

Bovine serum albumin .....	0.1g
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**Preparation of Bovine Serum Albumin Solution:**

Add bovine serum albumin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	0.4g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:**

Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.25g
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**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O Solution:**

Add Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components—except L-cysteine-HCl-H<sub>2</sub>O solution, Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O solution, and bovine serum albumin solution—to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 50°–55°C. Aseptically add the L-cysteine-HCl-H<sub>2</sub>O solution, Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O solution, and 10.0mL of sterile bovine serum albumin solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples.

**BCYE $\alpha$  without L-Cysteine  
(Buffered Charcoal Yeast  
Extract Agar without L-Cysteine)**

**Composition per liter:**

Agar .....	15.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	1.0g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O solution .....	10.0mL
pH 6.9 $\pm$ 0.2 at 25°C	

**Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.25g
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**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O Solution:**

Add Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust medium

to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile  $\text{Fe}_3(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$  solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples.

### ***Bdellovibrio* Medium**

**Composition** per Petri dish:

Base layer agar.....	10.0mL
Semisolid agar.....	10.0mL
Host medium.....	1.0mL

### **Host Medium:**

**Composition** per liter:

Yeast extract.....	3.0g
Peptone.....	0.6g

**Preparation of Host Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

### **Base Layer Agar:**

**Composition** per liter:

Agar.....	19.0g
Yeast extract.....	3.0g
Peptone.....	0.6g

**Preparation of Base Layer Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

### **Semisolid Agar:**

**Composition** per liter:

Agar.....	6.0g
Yeast extract.....	3.0g
Peptone.....	0.6g

**Preparation of Semisolid Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Inoculate appropriate bacterial host into 10.0mL of host medium. Hosts include *Erwinia amylovora*, *Escherichia coli*, *Serratia marcescens*, or *Pseudomonas putida*. Incubate host culture for 24–48 hr at 30°C. Melt the base layer agar and semisolid agar. Pour the base layer agar into a sterile Petri dish. Allow base layer agar to solidify. Cool the semisolid agar to 40°–45°C. Add 1.0mL of the previously grown host culture. Mix thoroughly. Pour over the solidified base layer agar.

**Use:** For the cultivation of *Bdellovibrio bacteriovorus* and *Bdellovibrio starrii*.

### **Beef Extract Agar**

**Composition** per liter:

Agar.....	15.0g
Peptone.....	5.0g
Beef extract.....	3.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Heat gently and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a wide variety of microorganisms. Recommended for the culture of microorganisms from water.

### **Beef Extract Broth**

**Composition** per liter:

Peptone.....	5.0g
Beef extract.....	3.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat gently and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of a wide variety of microorganisms. Recommended for the culture of microorganisms from water.

### ***Beggiatoa* and *Thiothrix* Medium**

**Composition** per liter:

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (saturated solution).....	20.0mL
$\text{NH}_4\text{Cl}$ (4% solution).....	5.0mL
Trace elements.....	5.0mL
$\text{K}_2\text{HPO}_4$ (1% solution).....	1.0mL
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1% solution).....	1.0mL

### **Trace Elements:**

**Composition** per liter:

EDTA solution.....	20.0mL
$\text{Co}(\text{NO}_3)_2$ (0.01% solution).....	10.0mL
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.00005% solution).....	10.0mL
$\text{H}_3\text{BO}_3$ (0.1% solution).....	10.0mL
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (0.02% solution).....	10.0mL
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.01% solution).....	10.0mL
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1% solution).....	10.0mL

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **EDTA Solution:**

**Composition** per 100.0mL:

$\text{FeSO}_4$ .....	7.0g
EDTA.....	2.0g
HCl, concentrated.....	1.0mL

**Preparation of EDTA Solution:** Add EDTA and  $\text{FeSO}_4$  to concentrated HCl. Mix thoroughly. Carefully add to distilled/deionized water and bring volume to 100.0mL.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Beggiatoa* species and myxotrophic *Thiothrix* species.

### ***Beggiatoa* Medium (ATCC Medium 138)**

**Composition** per liter:

Yeast extract.....	2.0g
Agar.....	2.0g
Sodium acetate.....	0.5g
$\text{CaCl}_2$ .....	0.1g
Catalase.....	10,000U

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except catalase, to tap water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10,000 units of sterile catalase.

**Use:** For the cultivation and maintenance of *Beggiatoa alba* and *Vitreoscilla* species.

### ***Beggiatoa* Medium (ATCC Medium 1193)**

**Composition** per liter:

Sodium sulfide .....	0.5g
Sodium acetate .....	0.01g
Yeast extract .....	0.01g
Nutrient broth .....	0.01g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Distribute into tubes or flasks.

**Use:** For the cultivation of *Beggiatoa alba*.

### ***Beijerinckia* Agar**

**Composition** per liter:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	5.0mg
ZnSO <sub>4</sub> ·6H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·6H <sub>2</sub> O .....	4.0mg
MnSO <sub>4</sub> ·6H <sub>2</sub> O .....	2.0mg
Glucose solution .....	50.0mL

pH 6.5 ± 0.2 at 25°C

**Glucose Solution:**

**Composition** per 50.0mL:

D-Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Beijerinckia derxii*, *Beijerinckia fluminensis*, *Beijerinckia indica*, *Beijerinckia mobilis*, *Beijerinckia* species, and *Clostridium barkeri*.

### ***Beijerinckia* Medium**

**Composition** per liter:

Glucose .....	20.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

pH 5.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Beijerinckia* species.

### ***Beijerinckia* Medium, Modified**

**Composition** per liter:

Agar .....	15.0g
Glucose .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.3mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	5.0mg

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Beijerinckia derxii*, *Beijerinckia fluminensis*, *Beijerinckia indica*, and *Beijerinckia mobilis*.

### ***Beijerinckia's Thiobacillus* Medium**

**Composition** per liter:

Noble agar .....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.2g
MgCl <sub>2</sub> .....	0.1g
NH <sub>4</sub> Cl .....	0.1g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution .....	100.0mL
NaHCO <sub>3</sub> solution .....	10.0mL

pH 7.0–7.2 at 25°C

**Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:**

**Composition** per 100.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	5.0g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and 10.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus thermophilica*.

### **Benzoate Medium**

**Composition** per liter:

Noble agar .....	20.0g
NaCl .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	3.0g
Sodium benzoate .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.2g
Yeast extract .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Benzoate solution .....	25.0mL

**Benzoate Solution:****Composition per 25.0mL:**

Sodium benzoate..... 3.0g

**Preparation of Benzoate Solution:** Add sodium benzoate to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components except benzoate solution to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Heat gently to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 25.0mL sterile benzoate solution. Mix thoroughly and pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Pseudomonas putida* and other microorganisms which can utilize benzoate as a carbon source.

**Benzoate Medium II****Composition per 1.5L:**

Noble agar..... 30.0g  
 (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>..... 3.0g  
 NaCl..... 1.67g  
 KH<sub>2</sub>PO<sub>4</sub>..... 1.2g  
 Yeast extract..... 0.5g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O..... 0.2g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O..... 0.1g  
 Benzoate solution..... 25.0mL

**Benzoate Solution:****Composition per 25.0mL:**

Sodium benzoate..... 1.0g

**Preparation of Benzoate Solution:** Add sodium benzoate to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except agar and sodium benzoate, to distilled/deionized water and bring volume to 600.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. In a separate flask, add agar to distilled/deionized water and bring volume to 375.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine the two autoclave-sterilized solutions. Mix thoroughly. Aseptically add the sterile benzoate solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Pseudomonas putida* and other microorganisms that can utilize benzoate as a carbon source.

**Benzoate Minimal Salts Medium****Composition per liter:**

K<sub>2</sub>HPO<sub>4</sub>..... 10.0g  
 NaNH<sub>4</sub>HPO<sub>4</sub>·4H<sub>2</sub>O..... 3.5g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O..... 0.2g  
 Citric acid, anhydrous..... 0.2g  
 Benzoate solution..... 25.0mL

pH 7.0 ± 0.2 at 25°C

**Benzoate Solution:****Composition per 25.0mL:**

Sodium benzoate..... 2.5g

**Preparation of Benzoate Solution:** Add sodium benzoate to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thor-

oughly. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically add 25.0mL of sterile benzoate solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of microorganisms that can utilize benzoate as a carbon source.

**Benzoate Nitrate Salts Medium (BNS)****Composition per liter:**

Solution A..... 700.0mL

Solution B..... 300.0mL

pH 8.2 ± 0.2 at 25°C

**Solution A:****Composition per 700.0mL:**

KNO<sub>3</sub>..... 2.0g  
 Sodium benzoate..... 1.0g  
 NH<sub>4</sub>Cl..... 0.3g  
 Phosphate buffer solution..... 200.0mL

**Phosphate Buffer Solution:****Composition per 200.0mL:**

K<sub>2</sub>HPO<sub>4</sub>..... 5.12g  
 KH<sub>2</sub>PO<sub>4</sub>..... 1.5g

**Preparation of Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Adjust pH to 9.0 with KOH.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 700.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Solution B:****Composition per 300.0mL:**

MgSO<sub>4</sub>·7H<sub>2</sub>O..... 0.2g  
 CaCl<sub>2</sub>..... 10.0mg  
 Trace metals solution..... 1.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 300.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Metals Solution:****Composition per 300.0mL:**

MnSO<sub>4</sub>·H<sub>2</sub>O..... 50.0mg  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O..... 50.0mg  
 Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O..... 10.0mg  
 CuSO<sub>4</sub>..... 10.0mg  
 Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O..... 10.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O..... 0.2mg  
 Ferric EDTA solution..... 10.0mL

**Preparation of Trace Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Ferric EDTA Solution:****Composition per 550.0mL:**

EDTA..... 17.9g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O..... 13.7g  
 KOH..... 3.23g

**Preparation of Ferric EDTA Solution:** Add EDTA and KOH to distilled/deionized water and bring volume to 186.0mL. Mix thoroughly. In a separate flask, add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 364.0mL. Mix thoroughly. Combine the two solutions. Sparge with air overnight to oxidize the Fe<sup>2+</sup> to Fe<sup>3+</sup>. Store in the dark.

**Preparation of Medium:** Aseptically combine 700.0mL of sterile solution A with 300.0mL of sterile solution B. Adjust pH to 8.2. Aseptically distribute into sterile screw-capped tubes. Fill tubes completely.

**Use:** For the cultivation of *Alcaligenes xylosoxydans*.

**BG 11 Agar**  
(Medium BG 11 for Cyanobacteria)

**Composition per liter:**

Agar .....	10.0g
NaNO <sub>3</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.075g
K <sub>2</sub> HPO <sub>4</sub> .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.036g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	6.0mg
Ferric ammonium citrate .....	6.0mg
Disodium EDTA .....	1.0mg
Trace metal mix A5 .....	1.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Metal Mix A5:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of Trace Metal Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat gently to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. For solid medium, pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a wide variety of cyanobacteria.

**BG 11 Marine Agar**  
(Medium BG 11 for Marine Cyanobacteria)

**Composition per liter:**

Agar .....	10.0g
NaCl .....	10.0g
NaNO <sub>3</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.075g
K <sub>2</sub> HPO <sub>4</sub> .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.036g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	6.0mg
Ferric ammonium citrate .....	6.0mg
EDTA disodium salt .....	1.0mg
Vitamin B <sub>12</sub> solution .....	100.0mL
Trace metal mix A5 .....	1.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Metal Mix A5:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of Trace Metal Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:**

**Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	1.0μg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Vitamin B<sub>12</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Heat gently to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Synechococcus* species. For the isolation of cyanobacteria from freshwater habitats.

**BG 11 Marine Broth**  
(Medium BG 11 for Marine Cyanobacteria)

**Composition per liter:**

NaCl .....	10.0g
NaNO <sub>3</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.075g
K <sub>2</sub> HPO <sub>4</sub> .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.036g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	6.0mg
Ferric ammonium citrate .....	6.0mg
EDTA disodium salt .....	1.0mg
Vitamin B <sub>12</sub> solution .....	100.0mL
Trace metal mix A5 .....	1.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Metal Mix A5:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of Trace Metal Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:**

**Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	1.0μg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Vitamin B<sub>12</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Heat gently to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Synechococcus* species. For the isolation of cyanobacteria from freshwater habitats.

**BG 11 Uracil Agar**

**Composition per liter:**

Agar .....	10.0g
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Uracil .....	2.8g
NaNO <sub>3</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.075g
K <sub>2</sub> HPO <sub>4</sub> .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.036g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	6.0mg
Ferric ammonium citrate .....	6.0mg
EDTA disodium salt .....	1.0mg
Trace metal mix A5 .....	1.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Metal Mix A5:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of Trace Metal Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat gently to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and maintenance of *Anabaena variabilis*.

**Bile Salts Gelatin Agar****Composition per 100.0mL:**

Gelatin .....	3.0g
Agar .....	1.5g
Pancreatic digest of casein .....	1.0g
NaCl .....	1.0g
Sodium taurocholate .....	0.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.1g
Water .....	100.0mL

pH 8.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Vibrio cholerae*.

**Biphenyl Agar  
(DSMZ Medium 457d)****Composition per liter:**

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
Biphenyl .....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements solution SL-4 .....	10.0mL

pH 6.9 ± 0.2 at 25°C

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Biphenyl Solution:****Composition per liter:**

Biphenyl .....	10.0g
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**Preparation of Biphenyl Solution:** Add biphenyl to 1.0L ethanol. Mix thoroughly. Filter sterilize using a cellulose filter membrane.

**Preparation of Medium:** Add components, except biphenyl solution, to 1.0L distilled/deionized water. Adjust pH to 6.9. Heat and gently bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Add an aliquot of the biphenyl solution to the lid of a sterile Petri dish so that the final concentration will be approximately 0.25g/L biphenyl, and let the ethanol evaporate so that the crystals of biphenyl coat the lid of the Petri dish. Aseptically add sterile agar medium to the crystal-layered Petri dish.

**Use:** For the cultivation of biphenyl-utilizing bacteria.

**Bismuth Sulfite Broth  
(m-Bismuth Sulfite Broth)****Composition per liter:**

Bi <sub>2</sub> (SO <sub>3</sub> ) <sub>3</sub> .....	16.0g
Pancreatic digest of casein .....	10.0g
Peptic digest of animal tissue .....	10.0g
Beef extract .....	10.0g
Glucose .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	8.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.6g

pH 7.7 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly and heat with frequent agitation until boiling. Boil for 1 min. Do not autoclave. Cool to 45°–50°C. Mix to disperse the precipitate and aseptically distribute into sterile tubes or flasks. Use 2.0–2.2mL of medium for each membrane filter.

**Use:** For the selective isolation of *Salmonella typhi* and other enteric bacilli and for the detection of *Salmonella* by the membrane filter method.

**Blastobacter denitrificans Agar  
(LMG Medium 157)****Composition per liter:**

Agar .....	15.0g
Tryptone .....	2.0g
Lab Lemco beef extract .....	0.5g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g
Glucose solution .....	10.0mL

pH 7.3 ± 0.2 at 25°C

**Glucose Solution:****Composition per 10.0mL:**

Glucose ..... 2.5g

**Preparation of Glucose Solution:** Add glucose to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add compents, except glucose solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL glucose solution. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Blastobacter denitrificans*.

**Blood Agar Base with 2.5% NaCl****Composition per liter:**

Beef heart, infusion from ..... 500.0g  
NaCl ..... 30.0g  
Agar ..... 15.0g  
Tryptose ..... 10.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat with frequent agitation and boil for 1 min to completely dissolve. Autoclave for 15 min at 15 psi pressure–121°C. Cool the basal medium to 45°–50°C. Aseptically add sterile, defibrinated blood to a final concentration of 5%. Mix thoroughly and pour into sterile Petri dishes.

**Use:** For the cultivation of *Paracoccus halodenitrificans*.

**Blood Glucose Cystine Agar****Composition per 100.0mL:**

Nutrient agar ..... 85.0mL  
Glucose cystine solution ..... 10.0mL  
Human blood, fresh ..... 5.0mL

pH 6.8 ± 0.2 at 25°C

**Nutrient Agar:****Composition per liter:**

Agar ..... 15.0g  
Pancreatic digest of gelatin ..... 5.0g  
Beef extract ..... 3.0g

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Nutrient Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Glucose Cystine Solution:****Composition per 50.0mL:**

Glucose ..... 12.5g  
L-Cystine-HCl ..... 0.5g

**Preparation of Glucose Cystine Solution:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 85.0mL of cooled, sterile agar solution, aseptically add 10.0mL of sterile glucose cystine solution and 5.0mL of human blood. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Francisella tularensis*.

**Blue-Green Agar****Composition per liter:**

Agar ..... 10.0g  
NaNO<sub>3</sub> ..... 1.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.075g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.04g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.036g  
Na<sub>2</sub>CO<sub>3</sub> ..... 0.02g  
Citric acid ..... 6.0mg  
Ferric ammonium citrate ..... 6.0mg  
EDTA disodium salt ..... 1.0mg  
Vitamin B<sub>12</sub> solution ..... 50.0mL  
Trace metal mix A5 ..... 1.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Metal Mix A5:****Composition per liter:**

H<sub>3</sub>BO<sub>3</sub> ..... 2.86g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.81g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.39g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.222g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.079g  
Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.049g

**Preparation of Trace Metal Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:****Composition per 50.0mL:**

Vitamin B<sub>12</sub> ..... 0.01g

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Vitamin B<sub>12</sub> solution, to glass-distilled water and bring volume to 950.0mL. Mix thoroughly. Heat gently and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool the basal medium to 45°–50°C. Add Vitamin B<sub>12</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Synechococcus* species.

**Blue-Green Nitrogen-Fixing Agar****Composition per liter:**

Noble agar ..... 10.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.075g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.04g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.036g  
Na<sub>2</sub>CO<sub>3</sub> ..... 0.02g  
Citric acid ..... 6.0mg  
Ferric ammonium citrate ..... 6.0mg  
EDTA disodium salt ..... 1.0mg  
Trace metal mix A5 ..... 1.0mL

pH 7.1 ± 0.2 at 25°C

**Trace Metal Mix A5:****Composition per liter:**

H<sub>3</sub>BO<sub>3</sub> ..... 2.86g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.81g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.39g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.222g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.079g  
Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.049g

**Preparation of Trace Metal Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to glass distilled water and bring volume to 1.0L. Mix thoroughly. Heat gently and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Check pH after autoclaving and readjust if necessary. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Calothrix*, *Fischerella*, and *Nostoc* species.

### **BMPA- $\alpha$ Medium (Edelstein BMPA- $\alpha$ Medium)**

**Composition per liter:**

Agar .....	13.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)-amino]-ethane sulfonic acid) .....	2.0g
Charcoal, activated .....	2.0g
$\alpha$ -Ketoglutarate .....	0.2g
$\text{Fe}_4(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$ .....	0.05g
Antibiotic inhibitor .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	10.0mL
pH $6.9 \pm 0.2$ at 25°C	

**Source:** This medium is available as premixed vials from Oxoid Unipath.

#### **Antibiotic Inhibitor:**

**Composition per 10.0mL:**

Anisomycin .....	0.08g
Cefamandole .....	4.0mg
Polymyxin B .....	80,000U

**Preparation of Antibiotic Inhibitor:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **L-Cysteine-HCl-H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	0.08g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cysteine and antibiotic inhibitor, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boiling for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 10.0mL of the sterile L-cysteine-HCl-H<sub>2</sub>O solution and 10.0mL of the sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the selective isolation and cultivation of *Legionella pneumophila* and other *Legionella* species.

### **BMS Agar**

**Composition per liter:**

Agar .....	20.0g
L-Malic acid .....	2.5g
Sucrose .....	2.5g
KOH .....	2.0g
Potato extract solution .....	950.0mL
Bromothymol Blue .....	1.0mL
Vitamin solution .....	1.0mL
pH $7.0 \pm 0.2$ at 25°C	

#### **Potato Extract Solution:**

**Composition per liter:**

Potatoes, washed, peeled, and sliced .....	200.0g
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**Preparation of Potato Extract Solution:** Wash, peel, and slice several large potatoes. Place the potato slices in a gauze bag. Place the bag with the potatoes in 1.0L of distilled/deionized water. Boil for 30 min. Filter through cotton.

#### **Bromothymol Blue Solution:**

**Composition per 100.0mL:**

Bromothymol Blue .....	0.5g
Ethanol, 95% .....	100.0mL

**Preparation of Bromothymol Blue Solution:** Add Bromothymol Blue to 100.0mL of 95% ethanol. Mix thoroughly.

#### **Vitamin Solution:**

**Composition per 100.0mL:**

Biotin .....	10.0g
Pyridoxine .....	20.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Dissolve 2.5g of L-malic acid in 50.0mL of distilled/deionized water. Add 1.0mL of Bromothymol Blue solution. Adjust pH to 7.0 by adding KOH so that the solution is green. Add 950.0mL of potato extract solution. Mix thoroughly. Add 20.0g of agar and 2.5g of sucrose. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 1.0mL of sterile vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Azospirillum lipoferum*.

### **Bosea thiooxidans Medium (DSMZ Medium 763)**

**Composition per liter:**

$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ .....	5.0g
Na-succinate .....	5.0g
$\text{Na}_2\text{HPO}_4$ .....	4.0g
$\text{KH}_2\text{PO}_4$ .....	1.5g
$\text{MgCl}_2$ .....	0.1g
Na-glutamate .....	0.5g
Yeast extract .....	0.1g
pH 7.5–8.5 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bosea thiooxidans*.

### **Bovine Albumin Tween 80 Medium, Ellinghausen and McCullough, Modified (Albumin Fatty Acid Broth, Leptospira Medium)**

**Composition per liter:**

Basal medium .....	900.0mL
Albumin fatty acid supplement .....	100.0mL

#### **Basal Medium:**

**Composition per liter:**

$\text{Na}_2\text{HPO}_4$ , anhydrous .....	1.0g
NaCl .....	1.0g
$\text{KH}_2\text{PO}_4$ , anhydrous .....	0.3g
$\text{NH}_4\text{Cl}$ (25% solution) .....	1.0mL
Glycerol (10% solution) .....	1.0mL

Sodium pyruvate (10% solution) .....	1.0mL
Thiamine-HCl (0.5% solution) .....	1.0mL
pH 7.4 ± 0.2 at 25°C	

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Albumin Fatty Acid Supplement:

##### Composition per 200.0mL:

Bovine albumin fraction V .....	20.0g
Polysorbate (Tween) 80 (10% solution) .....	25.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (0.5% solution) .....	20.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O (1.5% solution) .....	2.0mL
MgCl <sub>2</sub> ·2H <sub>2</sub> O (1.5% solution) .....	2.0mL
Vitamin B <sub>12</sub> (0.2% solution) .....	2.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.4% solution) .....	2.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.3% solution) .....	0.2mL

**Preparation of Albumin Fatty Acid Supplement:** Add bovine albumin to 100.0mL of distilled/deionized water. Mix thoroughly. Add remaining components while stirring. Adjust pH to 7.4. Bring volume to 200.0mL with distilled/deionized water. Filter sterilize. Store at –20°C.

**Preparation of Medium:** Aseptically combine 100.0mL of sterile albumin fatty acid supplement and 900.0mL of sterile basal medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leptospira* species.

#### Bovine Albumin Tween 80 Semisolid Medium, Ellinghausen and McCullough, Modified (Albumin Fatty Acid Semisolid Medium, Modified)

##### Composition per liter:

Basal medium .....	900.0mL
Albumin fatty acid supplement .....	100.0mL

##### Basal Medium:

##### Composition per liter:

Agar .....	2.2g
Na <sub>2</sub> HPO <sub>4</sub> , anhydrous .....	1.0g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> , anhydrous .....	0.3g
NH <sub>4</sub> Cl (25% solution) .....	1.0mL
Glycerol (10% solution) .....	1.0mL
Sodium pyruvate (10% solution) .....	1.0mL
Thiamine-HCl (0.5% solution) .....	1.0mL
pH 7.4 ± 0.2 at 25°C	

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Albumin Fatty Acid Supplement:

##### Composition per 200.0mL:

Bovine albumin fraction V .....	20.0g
Polysorbate (Tween) 80 (10% solution) .....	25.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (0.5% solution) .....	20.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O (1.5% solution) .....	2.0mL
MgCl <sub>2</sub> ·2H <sub>2</sub> O (1.5% solution) .....	2.0mL
Vitamin B <sub>12</sub> (0.2% solution) .....	2.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.4% solution) .....	2.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.3% solution) .....	0.2mL

#### Preparation of Albumin Fatty Acid Supplement:

Add bovine albumin to 100.0mL of distilled/deionized water. Mix thoroughly. Add remaining components while stirring. Adjust pH to 7.4. Bring volume to 200.0mL with distilled/deionized water. Filter sterilize. Store at –20°C.

**Preparation of Medium:** Aseptically combine 100.0mL of sterile albumin fatty acid supplement and 900.0mL of sterile basal medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leptospira* species.

#### Bovine Serum Albumin Tween 80 Agar (BSA Tween 80 Agar)

##### Composition per liter:

Basal medium .....	900.0mL
Albumin supplement .....	100.0mL

##### Basal Medium:

##### Composition per liter:

Agar .....	11.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
Glycerol (10% solution) .....	1.0mL
NH <sub>4</sub> Cl (25% solution) .....	1.0mL
Sodium pyruvate (10% solution) .....	1.0mL
Thiamine (0.5% solution) .....	1.0mL

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Albumin Supplement:

##### Composition per 100.0mL:

Bovine albumin .....	10.0g
Tween 80 (10% solution) .....	12.5mL
FeSO <sub>4</sub> (0.5% solution) .....	10.0mL
MgCl <sub>2</sub> -CaCl <sub>2</sub> solution .....	1.0mL
Cyanocobalamin (0.02% solution) .....	1.0mL
ZnSO <sub>4</sub> (0.4% solution) .....	1.0mL

**Preparation of Albumin Supplement:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.4. Filter sterilize.

#### MgCl<sub>2</sub>-CaCl<sub>2</sub> Solution:

##### Composition per 100.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.5g

**Preparation of MgCl<sub>2</sub>-CaCl<sub>2</sub> Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 900.0mL of cooled, sterile basal medium, aseptically add 100.0mL of sterile albumin supplement. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Leptospira* species.

#### Brackish Acetate

##### Composition per liter:

Sodium acetate .....	1.0g
KNO <sub>3</sub> .....	1.0g
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05g
Artificial seawater .....	250.0mL

Modified Hutner's basal salts .....	20.0mL
Vitamin solution.....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Artificial Seawater:****Composition per liter:**

NaCl .....	23.5g
MgCl <sub>2</sub> .....	5.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.9g
CaCl <sub>2</sub> .....	1.1g
KCl .....	0.66g
NaHCO <sub>3</sub> .....	0.19g
KBr .....	0.1g
H <sub>3</sub> BO <sub>3</sub> .....	0.026g
SrCl <sub>2</sub> .....	0.024g
NaF .....	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Modified Hutner's Basal Salts:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

**Preparation of Modified Hutner's Basal Salts:** Dissolve the nitrilotriacetic acid first and neutralize the solution with KOH. Add other components and adjust the pH to 7.2 with KOH or H<sub>2</sub>SO<sub>4</sub>. There may be a slight precipitate. Store at 5°C.

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile basal medium.

**Vitamin Solution:****Composition per liter:**

Thiamine·HCl .....	5.0mg
D-Calcium pantothenate .....	5.0mg
Riboflavin .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize and add aseptically to sterile basal medium.

**Preparation of Medium:** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to the distilled water to retard precipitation of the metal salts. Add components, except for "Metals 44" and vitamin solutions, to 250.0mL of artificial seawater and 720.0mL of distilled/deionized water. Adjust pH to 7.2. Distribute into tubes or flasks. Sterilize by autoclaving for 15 min at 15 lbs pressure–121°C. Aseptically add "Metals 44" and vitamin

solutions. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Filomicrobium fusiforme*.

### **Brackish Prosthecomicrobium Medium**

**Composition per liter:**

Agar .....	15.0g
Peptone .....	0.25g
Yeast extract .....	0.25g
Glucose .....	0.25g
Artificial seawater .....	250.0mL
Modified Hutner's basal salts .....	20.0mL
Vitamins .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Artificial Seawater:****Composition per liter:**

NaCl .....	23.477g
MgCl <sub>2</sub> .....	4.981g
Na <sub>2</sub> SO <sub>4</sub> .....	3.917g
CaCl <sub>2</sub> .....	1.102g
KCl .....	0.664g
NaHCO <sub>3</sub> .....	0.192g
KBr .....	0.096g
H <sub>3</sub> BO <sub>3</sub> .....	0.026g
SrCl <sub>2</sub> .....	0.024g
NaF .....	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Modified Hutner's Basal Salts:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

**Preparation of Modified Hutner's Basal Salts:**

Dissolve the nitrilotriacetic acid first and neutralize the solution with KOH. Add other ingredients and readjust the pH with KOH and/or H<sub>2</sub>SO<sub>4</sub> to 7.2. There may be a slight precipitate. Store at 5°C.

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile basal medium.

**Vitamin Solution:****Composition per liter:**

Thiamine·HCl .....	5.0mg
D-Calcium pantothenate .....	5.0mg
Riboflavin .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize and add aseptically to sterile basal medium.

**Preparation of Medium:** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to the distilled water to retard precipitation of the metal salts. Add components, except "Metals 44" and vitamin solutions, to 250.0mL of artificial seawater and 720.0mL of distilled/deionized water. Adjust pH to 7.2. Distribute into tubes or flasks. Sterilize by autoclaving for 15 min at 15 lbs pressure–121°C. Aseptically add "Metals 44" and vitamin solutions. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Prosthecomicrobium littoralum*.

#### Brain Heart Infusion with 3% Sodium Chloride

**Composition per liter:**

NaCl .....	30.0g
Pancreatic digest of gelatin .....	14.5g
Brain heart, solids from infusion .....	6.0g
Peptic digest of animal tissue .....	6.0g
Glucose .....	3.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	2.5g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Vibrio parahaemolyticus*.

#### Brain Heart Infusion Soil Extract Medium

**Composition per liter:**

Yeast extract .....	20.0g
Pancreatic digest of casein .....	16.0g
Brain heart, solids from infusion .....	8.0g
Peptic digest of animal tissue .....	5.0g
NaCl .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Glucose .....	2.0g
Soil extract .....	250.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Soil Extract:**

**Composition per 400.0mL:**

African Violet soil .....	1.0g
Na <sub>2</sub> CO <sub>3</sub> .....	1.0g

**Preparation of Soil Extract:** Autoclave for 60 min at 15 psi pressure–121°C. Filter through paper before using in medium.

**Vitamin B<sub>12</sub> Solution:**

**Composition per 1.0mL:**

Vitamin B <sub>12</sub> .....	2.0μg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose, yeast extract, and Vitamin B<sub>12</sub> solution, to tap water and bring volume to 799.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add yeast extract and glucose to 200.0mL of tap water. Filter sterilize and add aseptically to cooled, sterile basal medium. Aseptically add

1.0mL of Vitamin B<sub>12</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of a wide variety of microorganisms, including bacteria, yeasts, and molds, especially fastidious species from soil. For the isolation of *Histoplasma capsulatum* and other pathogenic fungi, including *Coccidioides immitis*, from soils.

#### Brevibacterium Medium (ATCC Medium 677)

**Composition per liter:**

Agar .....	30.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Yeast extract .....	2.0g
Tween™ 60 .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnSO <sub>4</sub> .....	0.01g
<i>n</i> -Hexadecane .....	50.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Blend for 30 min in a blender to disperse the *n*-hexadecane. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Brevibacterium alkanophilum* and other microorganisms that can utilize hexadecane as a carbon source.

#### Brevibacterium Medium (ATCC Medium 681)

**Composition per liter:**

Glucose .....	10.0g
Peptone .....	5.0g
Yeast extract .....	5.0g

pH 5.0–6.0 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Brevibacterium* spp. and *Enterobacter cloacae*.

#### Brevundimonas Agar (LMG Medium 221)

**Composition per liter:**

Agar .....	15.0g
Yeast extract .....	10.0g
Peptone .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.1g
Riboflavin solution .....	5.0mL

**Riboflavin Solution:**

**Composition per 10.0mL:**

Riboflavin .....	2.0mg
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**Preparation of Riboflavin Solution:** Add riboflavin to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except riboflavin, to distilled/deionized water and bring volume to 995.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to

45°–50°C. Aseptically add 5.0mL sterile riboflavin. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Brevundimonas* spp. and *Caulobacter henricii*.

### Brilliant Green Bile Agar

#### Composition per liter:

Noble agar .....	10.15g
Pancreatic digest of gelatin .....	8.25g
Lactose .....	1.9g
Na <sub>2</sub> SO <sub>3</sub> .....	0.205g
Basic Fuchsin .....	0.078g
Erioglaucine .....	0.065g
FeCl <sub>3</sub> .....	0.0295g
KH <sub>2</sub> PO <sub>4</sub> .....	0.015g
Oxgall, dehydrated .....	2.95mg
Brilliant Green .....	0.03mg

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contamination of the skin.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. For plating 10.0mL samples, prepare the medium double strength. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. Care should be taken to avoid exposure of the prepared medium to light.

**Use:** For the detection and enumeration of coliform bacteria in materials of sanitary importance such as water and sewage. *Escherichia coli* appears as dark red colonies with a pink halo. *Enterobacter* species appear as pink colonies.

### Brilliant Green Bile Broth (Brilliant Green Lactose Bile Broth)

#### Composition per liter:

Oxgall, dehydrated .....	20.0g
Lactose .....	10.0g
Pancreatic digest of gelatin .....	10.0g
Brilliant Green .....	0.013g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing inverted Durham tubes, in 10.0mL amounts for testing 1.0mL or less of sample. Autoclave for 12 min (not longer than 15 min) at 15 psi pressure–121°C. After sterilization, cool the broth rapidly. Medium is sensitive to light.

**Use:** For the detection of coliform microorganisms in water and wastewater as well as in other materials of sanitary importance. Turbidity in the broth and gas in the Durham tube are positive indications of *Escherichia coli*.

### Brilliant Green Bile Broth with MUG

#### Composition per liter:

Oxgall, dehydrated .....	20.0g
Lactose .....	10.0g
Pancreatic digest of gelatin .....	10.0g

MUG (4-Methyl umbelliferyl-

β-D-glucuronide) .....	0.05g
Brilliant Green .....	0.013g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing inverted Durham tubes, in 10.0mL amounts for testing 1.0mL or less of sample. Autoclave for 12 min (not longer than 15 min) at 15 psi pressure–121°C. After sterilization, cool the broth rapidly.

**Use:** For the detection of coliform microorganisms in water and wastewater, as well as in other materials of sanitary importance. The presence of *Escherichia coli* and other coliforms is determined by the presence of fluorescence in the tube.

### Brilliant Green Broth (m-Brilliant Green Broth)

#### Composition per liter:

Proteose peptone No. 3 .....	20.0g
Lactose .....	20.0g
Sucrose .....	20.0g
NaCl .....	10.0g
Yeast extract .....	6.0g
Phenol Red .....	0.16g
Brilliant Green .....	0.025g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat with frequent mixing. Boil for 1 min. Cool to 25°C. Add 2.0mL to each sterile absorbent filter used.

**Use:** For the selective isolation and differentiation of *Salmonella* from polluted water by the membrane filter method.

### Bromcresol Purple Broth

#### Composition per liter:

Peptone .....	10.0g
NaCl .....	5.0g
Beef extract .....	3.0g
Bromcresol Purple .....	0.04g
Carbohydrate solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

#### Carbohydrate Solution:

##### Composition per 10.0mL:

Carbohydrate .....	5.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into test tubes that contain an inverted Durham tube. Autoclave for 10 min at 15 psi pressure–121°C.

**Use:** For the differentiation of a variety of microorganisms based on their fermentation of specific carbohydrates. Bacteria that ferment the specific carbohydrate turn the medium yellow. When bacteria produce gas, the gas is trapped in the Durham tube.

### Bromcresol Purple Dextrose Broth (BCP Broth)

#### Composition per liter:

Glucose .....	10.0g
Peptone.....	5.0g
Beef extract.....	3.0g
Bromcresol Purple solution .....	2.0mL
pH 7.0 ± 0.2 at 25°C	

#### Bromcresol Purple Solution:

##### Composition per 10.0mL:

Bromcresol Purple .....	0.16g
Ethanol (95% solution).....	10.0mL

**Preparation of Bromcresol Purple Solution:** Add Bromcresol Purple to 10.0mL of ethanol. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes in 12–15mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria based on their ability to ferment glucose. Bacteria that ferment glucose turn the medium yellow.

### Bromothymol Blue Agar

#### Composition per liter:

Agar .....	11.0g
Peptone.....	10.0g
NaCl .....	5.0g
Yeast extract.....	5.0g
Lactose (33% solution).....	27.0mL
Bromothymol Blue (1% solution).....	10.0mL
Sodium thiosulfate (50% solution).....	2.0mL
Glucose (33% solution).....	1.2mL
Maranil solution (5% solution).....	1.0mL
pH 7.7–7.8 at 25°C	

**Preparation of Medium:** Add agar, peptone, NaCl, and yeast extract to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.0. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 45°–50°C. Filter sterilize separately the lactose solution, Bromothymol Blue solution, sodium thiosulfate solution, glucose solution, and maranil solution. To the cooled, sterile agar solution aseptically add 27.0mL of sterile lactose solution, 10.0mL of sterile Bromothymol Blue solution, 2.0mL of sterile sodium thiosulfate solution, 1.2mL of sterile glucose solution and 1.0mL of sterile maranil solution. Mix thoroughly. Adjust pH to 7.7–7.8. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of members of the *Enterobacteriaceae*.

### BSR Medium

#### Composition per liter:

Beef heart, solids from infusion.....	500.0g
Sorbitol.....	70.0g
Sucrose.....	10.0g
Tryptose .....	10.0g
NaCl .....	5.0g
Fructose.....	1.0g
Glucose .....	1.0g
Phenol Red.....	0.02g
Horse serum .....	100.0mL
pH 7.6 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except horse serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi

pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of horse serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Spiroplasma citri*.

### BSTSY Agar

#### Composition per liter:

Pancreatic digest of casein .....	17.0g
Agar .....	15.0g
NaCl .....	5.0g
Yeast extract .....	4.0g
Papaic digest of soybean meal .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Glucose.....	2.5g
Bovine serum.....	100.0mL
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except bovine serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile bovine serum. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Simonsiella* species and *Alysiiella* species.

### Buffered Charcoal Yeast Extract Differential Agar (DIFF/BCYE)

#### Composition per 1014.0mL:

Agar .....	17.0g
ACES (2-[(2-Amino-2-oxoethyl)- amino]-ethane sulfonic acid) buffer.....	10.0g
Yeast extract .....	10.0g
Charcoal, activated .....	1.5g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.25g
Bromcresol Purple.....	0.01g
Bromothymol Blue.....	0.01g
Antibiotic solution.....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution.....	4.0mL
pH 6.9 ± 0.2 at 25°C	

#### Antibiotic Solution:

##### Composition per 10.0mL:

Vancomycin.....	1.0mg
Polymyxin B.....	50,000U

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### L-Cysteine·HCl·H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

L-Cysteine·HCl·H <sub>2</sub> O .....	1.0g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine·HCl·H<sub>2</sub>O solution and antibiotic solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boil for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution and 10.0mL of sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.



**Use:** For the isolation, cultivation, and maintenance of *Legionella pneumophila* and other *Legionella* species from environmental samples. For the selective recovery of *Legionella pneumophila* while reducing contaminating microorganisms from environmental water samples.

#### Buffered Marine Yeast Medium

##### Composition per liter:

NaCl .....	24.0g
Agar .....	20.0g
Yeast extract .....	5.0g
1M Phosphate buffer, pH 6.8 .....	20.0mL
Hutner's mineral base .....	20.0mL
KOH (1N) .....	7.0mL

pH 6.8 ± 0.2 at 25°C

#### 1M Phosphate Buffer, pH 6.8:

##### Composition per liter:

K <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> .....	85.4g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	70.4g

**Preparation of 1M Phosphate Buffer, pH 6.8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8.

#### Hutner's Mineral Base:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

**Preparation of Hutner's Mineral Base:** Initially add a few drops of H<sub>2</sub>SO<sub>4</sub> to the distilled water to retard precipitation. Dissolve the nitrilotriacetic acid first and neutralize the solution with KOH. Add other ingredients and adjust the pH to 7.2 with KOH and/or H<sub>2</sub>SO<sub>4</sub>. There may be a slight precipitate. Store at 5°C.

#### "Metals 44":

##### Composition per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add aseptically to sterile basal medium.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

#### Burke's Modified Nitrogen-Free Medium

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.19g
NaHCO <sub>3</sub> .....	0.05g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.02g
KH <sub>2</sub> PO <sub>4</sub> .....	0.011g

SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g
NaCl .....	0.01g
Adenine .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.0mg
Na <sub>2</sub> MoO <sub>3</sub> .....	0.5mg

pH 7.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Azotobacter vinelandii*.

#### Burke's Modified Nitrogen-Free Medium with Benzoate

##### Composition per liter:

Sodium benzoate .....	0.72g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.189g
NaHCO <sub>3</sub> .....	0.05g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.02g
KH <sub>2</sub> PO <sub>4</sub> .....	0.011g
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g
NaCl .....	0.01g
Adenine .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.0mg
Na <sub>2</sub> MoO <sub>3</sub> .....	0.5mg

pH 7.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas* species and other microorganisms which can utilize benzoate as sole carbon source.

#### Bushnell-Haas Agar

##### Composition per liter:

Agar .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. For use in cultivating hydrocarbon-utilizing bacteria, layer 0.1–1.0% hydrocarbon on agar surface or aseptically add sterile hydrocarbon to cooled agar prior to pouring plates.

**Use:** For examining fuels for microbial contamination and for studying hydrocarbon utilization by microorganisms. Also for the cultivation of *Nocardia* species.

#### Butanediol Medium

##### Composition per liter:

NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	2.1g
1,4-Butanediol .....	1.0g
NaCl .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
Yeast extract.....	0.2g
Modified Wolfe's mineral solution.....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Modified Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.01g
NaWO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.01g

**Preparation of Modified Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas putida*.

***Butyrivibrio* species Medium****Composition per 1001.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
Pancreatic digest of casein.....	2.0g
Yeast extract.....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
Hemin.....	1.0mg
Resazurin.....	1.0mg
Rumen fluid, clarified.....	150.0mL
Minerals solution.....	75.0mL
Carbohydrate solution.....	20.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Volatile fatty acid mixture.....	3.1mL
pH 6.7 ± 0.2 at 25°C	

**Minerals Solution:****Composition per liter:**

NaCl.....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.6g

**Preparation of Minerals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**L-Cysteine-HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O.....	0.25g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Carbohydrate Solution:****Composition per 20.0mL:**

Glucose.....	1.0g
Cellobiose.....	1.0g
Glycerol.....	1.0g
Maltose.....	1.0g
Starch, soluble.....	1.0g

**Preparation of Carbohydrate Solution:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge under 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Volatile Fatty Acid Mixture:****Composition per 7.75mL:**

Acetic acid.....	4.25mL
Propionic acid.....	1.50mL
Butyric acid.....	1.0mL
DL-2-Methyl butyric acid.....	0.25mL
iso-Butyric acid.....	0.25mL
iso-Valeric acid.....	0.25mL
n-Valeric acid.....	0.25mL

**Preparation of Volatile Fatty Acid Mixture:** Combine components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% CO<sub>2</sub>. Add components, except carbohydrate solution, Na<sub>2</sub>CO<sub>3</sub>, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 100% CO<sub>2</sub>. Add Na<sub>2</sub>CO<sub>3</sub>. Continue sparging with 100% CO<sub>2</sub> until pH reaches 6.8. Distribute into rubber-stoppered tubes under 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile carbohydrate solution, 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution or, using a syringe, inject the appropriate amount of sterile carbohydrate solution, sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and sterile L-cysteine-HCl·H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation of *Butyrivibrio* species.

**BY Agar Medium  
(ATCC Medium 2038)****Composition per liter:**

Agar.....	15.0g
Yeast extract.....	5.0g
Pancreatic digest of casein.....	5.0g
Beef extract.....	5.0g
NaCl.....	2.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Paracoccus thiocyanatus*.

### BYEB

#### (Buffered Yeast Extract Broth)

##### Composition per liter:

ACES buffer (2-[(2-Amino-2-oxoethyl)-amino]-ethane sulfonic acid) .....	10.0g
Yeast extract .....	10.0g
$\alpha$ -Ketoglutarate .....	1.0g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.4g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.25g
pH 6.9 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.9. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Legionella pneumophila*.

### C/10 Agar

##### Composition per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.36g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Cytophaga flevensis*, *Flexibacter filiformis*, *Myxococcus fulvus*, and *Myxococcus xanthus*.

### C 3G *Spiroplasma* Medium

##### Composition per liter:

Sucrose .....	100.0g
Phenol Red .....	10.0mg
PPLO broth without Crystal Violet .....	500.0mL
Horse serum .....	150.0mL
Fresh yeast extract solution .....	50.0mL
CMRL-1066 medium .....	5.0mL
pH 7.5 ± 0.2 at 25°C	

**Source:** PPLO broth without Crystal Violet is available as a premixed powder from BD Diagnostic Systems.

##### PPLO Broth without Crystal Violet:

###### Composition per 500.0mL:

Beef heart, infusion from .....	11.52g
Peptone .....	2.32g
NaCl .....	1.15g

**Preparation of PPLO Broth without Crystal Violet:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

##### Fresh Yeast Extract Solution:

###### Composition per 100.0mL:

Baker's yeast, live, pressed, starch-free .....	25.0g
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**Preparation of Fresh Yeast Extract Solution:** Add the live Baker's yeast to 100.0mL of distilled/deionized water. Autoclave for 90 min at 15 psi pressure–121°C. Allow to stand. Remove supernatant solution. Adjust pH to 6.6–6.8. Filter sterilize.

### CMRL-1066 Medium:

##### Composition per liter:

NaCl .....	6.8g
NaHCO <sub>3</sub> .....	2.2g
D-Glucose .....	1.0g
KCl .....	0.4g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.26g
CaCl <sub>2</sub> , anhydrous .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	0.14g
L-Glutamine .....	0.1g
Sodium acetate·3H <sub>2</sub> O .....	0.083g
L-Glutamic acid .....	0.075g
L-Arginine-HCl .....	0.07g
L-Lysine-HCl .....	0.07g
L-Leucine .....	0.06g
Glycine .....	0.05g
Ascorbic acid .....	0.05g
L-Proline .....	0.04g
L-Tyrosine .....	0.04g
L-Aspartic acid .....	0.03g
L-Threonine .....	0.03g
L-Alanine .....	0.025g
L-Phenylalanine .....	0.025g
L-Serine .....	0.025g
L-Valine .....	0.025g
L-Cystine .....	0.02g
L-Histidine-HCl-H <sub>2</sub> O .....	0.02g
L-Isoleucine .....	0.02g
Phenol Red .....	0.02g
L-Methionine .....	0.015g
Deoxyadenosine .....	0.01g
Deoxycytidine .....	0.01g
Deoxyguanosine .....	0.01g
Glutathione, reduced .....	0.01g
Thymidine .....	0.01g
Hydroxy-L-proline .....	0.01g
L-Tryptophan .....	0.01g
Nicotinamide adenine dinucleotide .....	7.0mg
Tween 80 .....	5.0mg
Sodium glucuronate-H <sub>2</sub> O .....	4.2mg
Coenzyme A .....	2.5mg
Coccarboxylase .....	1.0mg
Flavin adenine dinucleotide .....	1.0mg
Nicotinamide adenine dinucleotide phosphate .....	1.0mg
Uridine triphosphate .....	1.0mg
Choline chloride .....	0.5mg
Cholesterol .....	0.2mg
5-Methyldeoxycytidine .....	0.1mg
Inositol .....	0.05mg
<i>p</i> -Aminobenzoic acid .....	0.05mg
Niacin .....	0.025mg
Niacinamide .....	0.025mg
Pyridoxine .....	0.025mg
Pyridoxal-HCl .....	0.025mg
Biotin .....	0.01mg
D-Calcium pantothenate .....	0.01mg
Folic acid .....	0.01mg
Riboflavin .....	0.01mg
Thiamine-HCl .....	0.01mg

**Source:** CMRL-1066 medium is available as a premixed powder from BD Diagnostics.

**Preparation of CMRL-1066 Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Filter sterilize.

**Preparation of Medium:** Add components—except horse serum, fresh yeast extract, and CMRL medium—to distilled/deionized water and bring volume to 795.0mL. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure—121°C. Aseptically add 150.0mL of sterile horse serum, 50.0mL of sterile fresh yeast extract solution, and 5.0mL of sterile CMRL medium. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma* species.

### C 3N *Spiroplasma* Medium

**Composition** per 100.0mL:

Sucrose	12.0g
Phenol Red	10.0mg
PPLO broth without Crystal Violet	50.0mL
Horse serum	20.0mL
Fresh yeast extract solution	5.0mL
CMRL-1066 medium	0.5mL

pH 7.5 ± 0.2 at 25°C

### PPLO Broth without Crystal Violet:

**Composition** per 500.0mL:

Beef heart, infusion from	11.52g
Peptone	2.32g
NaCl	1.15g

**Source:** PPLO broth without Crystal Violet is available as a premixed powder from BD Diagnostic Systems.

**Preparation of PPLO Broth without Crystal Violet:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

### Fresh Yeast Extract Solution:

**Composition** per 100.0mL:

Baker's yeast, live, pressed, starch-free	25.0g
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**Preparation of Fresh Yeast Extract Solution:** Add the live Baker's yeast to 100.0mL of distilled/deionized water. Autoclave for 90 min at 15 psi pressure—121°C. Allow to stand. Remove supernatant solution. Adjust pH to 6.6–6.8. Filter sterilize.

### CMRL-1066 Medium:

**Composition** per liter:

NaCl	6.8g
NaHCO <sub>3</sub>	2.2g
D-Glucose	1.0g
KCl	0.4g
L-Cysteine-HCl·H <sub>2</sub> O	0.26g
CaCl <sub>2</sub> , anhydrous	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	0.14g
L-Glutamine	0.1g
Sodium acetate·3H <sub>2</sub> O	0.083g
L-Glutamic acid	0.075g
L-Arginine-HCl	0.07g
L-Lysine-HCl	0.07g
L-Leucine	0.06g
Glycine	0.05g
Ascorbic acid	0.05g
L-Proline	0.04g
L-Tyrosine	0.04g
L-Aspartic acid	0.03g
L-Threonine	0.03g
L-Alanine	0.025g
L-Phenylalanine	0.025g
L-Serine	0.025g
L-Valine	0.025g
L-Cystine	0.02g

L-Histidine-HCl·H <sub>2</sub> O	0.02g
L-Isoleucine	0.02g
Phenol Red	0.02g
L-Methionine	0.015g
Deoxyadenosine	0.01g
Deoxycytidine	0.01g
Deoxyguanosine	0.01g
Glutathione, reduced	0.01g
Thymidine	0.01g
Hydroxy-L-proline	0.01g
L-Tryptophan	0.01g
Nicotinamide adenine dinucleotide	7.0mg
Tween 80	5.0mg
Sodium glucuronate·H <sub>2</sub> O	4.2mg
Coenzyme A	2.5mg
Coccarboxylase	1.0mg
Flavin adenine dinucleotide	1.0mg
Nicotinamide adenine dinucleotide phosphate	1.0mg
Uridine triphosphate	1.0mg
Choline chloride	0.5mg
Cholesterol	0.2mg
5-Methyldeoxycytidine	0.1mg
Inositol	0.05mg
p-Aminobenzoic acid	0.05mg
Niacin	0.025mg
Niacinamide	0.025mg
Pyridoxine	0.025mg
Pyridoxal-HCl	0.025mg
Biotin	0.01mg
D-Calcium pantothenate	0.01mg
Folic acid	0.01mg
Riboflavin	0.01mg
Thiamine-HCl	0.01mg

**Source:** CMRL-1066 medium is available as a premixed powder from BD Diagnostics.

**Preparation of CMRL-1066 Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Filter sterilize.

**Preparation of Medium:** Add components—except horse serum, fresh yeast extract, and CMRL medium—to distilled/deionized water and bring volume to 75.0mL. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure—121°C. Aseptically add 20.0mL of sterile horse serum, 5.0mL of sterile yeast extract, and 0.5mL of sterile CMRL medium. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma kunkelii*.

### CAL Agar (Cellobiose Arginine Lysine Agar) (Yersinia Isolation Agar)

**Composition** per liter:

Agar	20.0g
L-Arginine-HCl	6.5g
L-Lysine-HCl	6.5g
NaCl	5.0g
Cellobiose	3.5g
Yeast extract	3.0g
Sodium deoxycholate	1.5g
Neutral Red	0.03g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat to boiling. Do not autoclave. Pour into sterile Petri dishes.

**Use:** For the isolation and characterization of *Yersinia enterocolitica* and enumeration of *Yersinia enterocolitica* from water and other liquid specimens.

### *Caldicellulosiruptor Medium*

#### **Composition** per liter:

Pancreatic digest of casein	2.0g
K <sub>2</sub> HPO <sub>4</sub>	1.5g
Cellobiose	1.0g
Yeast extract	1.0g
NaCl	0.9g
NH <sub>4</sub> Cl	0.9g
KH <sub>2</sub> PO <sub>4</sub>	0.75g
L-Cysteine-HCl	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.4g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	2.5mg
Resazurin	0.5mg
Trace elements solution SL-10	1.0mL

pH 7.2 ± 0.2 at 25°C

#### **Trace Elements Solution SL-10:**

##### **Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Caldicellulosiruptor saccharolyticus*.

### *Caldicellulosiruptor Medium*

#### **Composition** per 1001.0mL:

Pancreatic digest of casein	2.0g
K <sub>2</sub> HPO <sub>4</sub>	1.5g
Cellulose	1.0g
Cellobiose	1.0g
Yeast extract	1.0g
NaCl	0.9g
NH <sub>4</sub> Cl	0.9g
KH <sub>2</sub> PO <sub>4</sub>	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.4g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	2.5mg
L-Cysteine-HCl	0.75g
Resazurin	0.5mg

pH 7.2 ± 0.2 at 25°C

#### **Trace Elements Solution SL-10:**

##### **Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg

H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Caldicellulosiruptor saccharolyticus*.

### *Camphor Minimal Medium*

#### **Composition** per liter:

Agar	20.0g
K <sub>2</sub> HPO <sub>4</sub>	4.4g
NH <sub>4</sub> Cl	2.1g
KH <sub>2</sub> PO <sub>4</sub>	1.7g
100× salt solution	10.0mL

#### **100× Salt Solution:**

##### **Composition** per liter:

MgSO <sub>4</sub>	19.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	5.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O	5.0g
Ascorbic acid	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.3g

**Preparation of 100× Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. Allow to cool to room temperature. Invert Petri dishes. Spread 0.2mL of 2m D-(+) camphor solution in methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) on the inside cover of each plate.

**Use:** For the cultivation and maintenance of *Pseudomonas putida*.

### *Carbohydrate Fermentation Broth*

#### **Composition** per liter:

Peptone	10.0g
NaCl	5.0g
Meat extract	3.0g
Carbohydrate solution	50.0mL
Andrade's indicator	10.0mL

pH 7.1 ± 0.2 at 25°C

#### **Andrade's Indicator:**

##### **Composition** per 100.0mL:

Acid Fuchsin	0.1g
NaOH (1N solution)	16.0mL

**Preparation of Andrade's Indicator:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **Carbohydrate Solution:**

##### **Composition** per 100.0mL:

Carbohydrate	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to

100.0mL. Adonitol, arabinose, cellobiose, glucose, dulcitol, fructose, galactose, inositol, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, xylose, or other carbohydrates may be used. Mix thoroughly. Filter sterilize.

**Caution:** Acid Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute in 10.0mL volumes into test tubes containing inverted Durham tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Add 0.5mL of sterile carbohydrate solution to each tube.

**Use:** For the determination of carbohydrate fermentation reactions of microorganisms, particularly members of the Enterobacteriaceae. A Durham tube is used to collect gas produced during the fermentation reaction. Acid production is indicated by a pink reaction.

### Carbon Monoxide Oxidizers Agar, Modified

**Composition per 1001.0mL:**

Agar .....	12.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	4.5g
Sodium acetate .....	3.0g
NH <sub>4</sub> Cl .....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	30.0mg
Ferric ammonium citrate .....	18.0mg
Trace elements solution .....	1.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Thiamine-HCl solution .....	10.0mL

### Trace Elements Solution:

**Composition per liter:**

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.9g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	20.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### NaHCO<sub>3</sub> Solution:

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Filter sterilize.

### Thiamine-HCl Solution:

**Composition per 10.0mL:**

Thiamine-HCl .....	20.0μg
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**Preparation of Thiamine-HCl Solution:** Add thiamine-HCl to distilled/deionized water and bring volume to 10.0mL. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution and thiamine-HCl solution, to distilled/deionized water and bring volume to 980.0mL. Mix thor-

oughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile NaHCO<sub>3</sub> solution and 10.0mL of sterile thiamine-HCl solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Carbophilus carboxidus* and *Zavarzinia compransoris*.

### Carbon Utilization Test

**Composition per liter:**

Ionagar .....	10.0g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Ferric ammonium citrate .....	0.05g
CaCl <sub>2</sub> .....	0.5mg
Sodium potassium phosphate buffer (0.33M solution, pH 6.8) .....	1.0L
Carbon source .....	10.0mL
pH 6.8 ± 0.2 at 25°C	

### Carbon Source:

**Composition per 10.0mL:**

Carbon source .....	1.0g
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**Preparation of Carbon Source:** Add carbon source to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbon source, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile carbon source. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and differentiation of *Pseudomonas* species based on their ability to utilize a specific carbon source.

### Carbonate-Buffered Medium CMB4 with Glucose

**Composition per 1002.0mL:**

NaCl .....	4.0g
NaHCO <sub>3</sub> .....	2.5g
Glucose .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.8g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Resazurin .....	1.0mg
Modified Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Sulfide-calcium solution .....	2.0mL
pH 6.9 ± 0.2 at 25°C	

### Modified Wolfe's Mineral Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

Na <sub>2</sub> SeO <sub>3</sub> .....	0.01g
NaWO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Modified Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### Wolfe's Vitamin Solution:

**Composition per liter:**

Pyridoxine·HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Sulfide-Calcium Solution:

**Composition per liter:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	36.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	15.0g

**Preparation of Sulfide-Calcium Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Sparge with 100% N<sub>2</sub>. Anaerobically distribute into tubes. Autoclave at 121°C for 15 min.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and sulfide-calcium solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Anaerobically distribute 10.0mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.02mL of sterile sulfide-calcium solution to each tube. Mix thoroughly. Adjust pH to 6.9.

**Use:** For the cultivation of *Spirochaeta thermophila*.

#### *Carboxydobacterium* Medium

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	20.0mg
Ferric ammonium citrate .....	1.2mg
TS2 trace elements solution .....	1.0mL

#### TS2 Trace Elements Solution:

**Composition per liter:**

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.9g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	20.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of TS2 Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Caution:** Carbon monoxide (CO) is a toxic gas.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. After inoculation, incubate in an atmosphere of 50% CO + 50% air.

**Use:** For the autotrophic cultivation of *Oligotropha carboxidovorans*.

#### *Carboxydobacterium* Medium

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
Sodium acetate .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	20.0mg
Ferric ammonium citrate .....	1.2mg
TS2 trace elements solution .....	1.0mL

#### TS2 Trace Elements Solution:

**Composition per liter:**

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.9g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	20.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of TS2 Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. After inoculation, incubate in air.

**Use:** For the organotrophic cultivation of *Oligotropha carboxidovorans*.

#### *Carboxydotherrmus* Medium

**Composition per 1030.0mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.52g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
NH <sub>4</sub> Cl .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.29g
Resazurin .....	0.5mg
Trace elements solution SL-4 .....	10.0mL
Vitamin solution .....	10.0mL
Yeast extract solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 6.9 ± 0.2 at 25°C

#### Trace Elements Solution SL-4:

**Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Trace Elements Solution SL-6:

## Composition per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.5g
H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Vitamin Solution:

## Composition per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

# Yeast Extract Solution:

## Composition per 10.0mL:

Yeast extract	10.0mg
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

# Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

## Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.7g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Prepare and dispense solution anaerobically under 100% N<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Adjust pH to 7.0. Autoclave for 15 min at 15 psi–121°C.

**Preparation of Medium:** Add components, except vitamin solution, yeast extract solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge under 100% N<sub>2</sub> for 10 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically combine with 10.0mL of sterile vitamin solution, 10.0mL of sterile yeast extract solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Pressurize inoculation flask with CO (carbon monoxide) gas at 2 bar pressure.

**Caution:** Carbon monoxide is a toxic gas.

**Use:** For the cultivation and maintenance of *Carboxydot-hermus hydrogenofmans*.

## *Carboxydobrachium pacificum* Medium (DSMZ Medium 902)

## Composition per 1050mL:

NaCl	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.9g
KCl	0.7g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.4g

NH <sub>4</sub> Cl	0.3g
Na <sub>2</sub> HPO <sub>4</sub>	0.15g
Yeast extract	0.05g
Na <sub>2</sub> SiO <sub>3</sub>	0.03g
Resazurin	0.5mg
Vitamin solution	10.0mL
NaHCO <sub>3</sub> solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
L-Cysteine solution	10.0mL
Pyruvate solution	10.0mL
Trace elements solution SL-10	1.0mL
pH 7.0 ± 0.2 at 25°C	

# Pyruvate Solution:

## Composition per 10.0mL:

Na-pyruvate	2.5g
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**Preparation of Pyruvate Solution:** Add pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# L-Cysteine Solution:

## Composition per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O	0.45g
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**Preparation of L-Cysteine Solution:** Add L-cys-teine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

## Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.45g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# NaHCO<sub>3</sub> Solution:

## Composition per 10.0mL:

NaHCO <sub>3</sub>	0.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

# Trace Elements Solution SL-10:

## Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

# Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Vitamin Solution:

## Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg



<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, pyruvate solution, L-Cysteine-HCl-H<sub>2</sub>O solution, and Na<sub>2</sub>S-9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Sparge with 100% N<sub>2</sub> of at least 30 min. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter, 10.0mL vitamin solution, 10.0mL NaHCO<sub>3</sub> solution, 10.0mL pyruvate solution, 10.0mL L-Cysteine-HCl-H<sub>2</sub>O solution, and 10.0mL Na<sub>2</sub>S-9H<sub>2</sub>O. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Carboxydibrachium pacificum* (*Carboxydibrachium pacificum*).

### *Caryophanon latum* Medium

**Composition** per liter:

Papaic digest of soybean meal .....	2.0g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Sodium acetate .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.27g
Sodium glutamate .....	0.1g
Thiamine-HCl .....	0.2mg
Biotin .....	0.05mg
Tris/HCl-buffer 10mM, pH 7.8 .....	1000.0mL
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Combine components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Caryophanon latum* and *Vitreoscilla stercoraria*.

### CAS Medium

**Composition** per liter:

Pancreatic digest of casein .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of myxobacteria.

### Casamino Acids Glucose Medium (CAGV Medium)

**Composition** per liter:

Agar .....	20.0g
Glucose .....	1.0g
Vitamin-free casamino acids.....	1.0g
Solution A (Mineral Salts).....	20.0mL
Vitamin solution.....	10.0mL
pH 7.2 ± 0.2 at 25°C	

### Solution A (Mineral Salts):

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	12.67g
Nitritotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Solution B (Metallic salts).....	50.0mL

**Preparation of Solution A (Mineral Salts):** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L.

### Solution B (Metallic Salts):

**Composition** per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Ethylenediaminetetraacetic acid.....	0.3g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.3g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.02g

**Preparation of Solution B (Metallic Salts):** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	0.01g
Calcium pantothenate .....	5.0mg
Nicotinamide .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Microcycilus aquaticus*.

### Casein Medium

**Composition** per liter:

NaCl .....	250.0g
Agar .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0g
Casein hydrolysate .....	5.0g
Yeast extract .....	5.0g
KCl .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
pH 7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat to boiling. Adjust pH to 7.4. Bring volume to 1.0L with distilled/deionized water. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Halobacterium* species and other halophilic bacteria.

### Casitone Agar

**Composition** per liter:

Pancreatic digest of casein .....	20.0g
Agar .....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Potassium phosphate buffer (0.01M solution, pH 7.2) .....	1.0L
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat to boiling. Distribute into tubes or flasks. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Myxococcus* species.

### Casitone Glycerol Yeast Autolysate Broth (CGY Autolysate Broth)

**Composition** per liter:

Pancreatic digest of casein .....	5.0g
Yeast autolysate .....	1.0g
Glycerol .....	10.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enumeration, of iron and sulfur bacteria from the *Sphaerotilus* group.

### Castenholz D Medium (Medium D)

**Composition** per liter:

NaNO <sub>3</sub> .....	0.7g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06g
NaCl .....	8.0mg
FeCl <sub>3</sub> solution .....	1.0mL
Micronutrient solution .....	0.5mL
pH 7.5 ± 0.2 at 25°C	

### FeCl<sub>3</sub> Solution:

**Composition** per liter:

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.28g
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Micronutrient Solution:

**Composition** per liter:

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.28g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

**Preparation of Micronutrient Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Readjust pH to 7.5. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation of cyanobacteria, including thermophilic species. For the cultivation of *Chloroflexus* species and *Fischerella* species.

### Castenholz D Medium, Modified (Medium D, Modified)

**Composition** per liter:

NaCl .....	160.0g
NaNO <sub>3</sub> .....	0.69g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.111g
KNO <sub>3</sub> .....	0.103g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06g
FeCl <sub>3</sub> .....	0.3mg
Trace metal solution, Castenholz .....	1.0mL
pH 7.5 ± 0.2 at 25°C	

### Trace Metal Solution, Castenholz:

**Composition** per liter:

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.28g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

### Preparation of Trace Metal Solution, Castenholz:

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Readjust pH to 7.5. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute into screw-capped tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation of halophilic cyanobacteria.

### Castenholz DG Medium (Medium DG)

**Composition** per liter:

Glycyl-glycine buffer .....	0.8g
NaNO <sub>3</sub> .....	0.7g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06g
NaCl .....	8.0mg
FeCl <sub>3</sub> solution .....	1.0mL
Micronutrient solution .....	0.5mL
pH 7.5 ± 0.2 at 25°C	

### FeCl<sub>3</sub> Solution:

**Composition** per liter:

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.28g
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Micronutrient Solution:****Composition** per liter:

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.28g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

**Preparation of Micronutrient Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Readjust pH to 8.1. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation of cyanobacteria, including thermophilic species.

### **Castenholz DGN Medium (Medium DGN)**

**Composition** per liter:

Glycyl-glycine buffer .....	0.8g
NaNO <sub>3</sub> .....	0.7g
NH <sub>4</sub> Cl .....	0.2g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06g
NaCl .....	8.0mg
FeCl <sub>3</sub> solution .....	1.0mL
Micronutrient solution .....	0.5mL

pH 7.5 ± 0.2 at 25°C

**FeCl<sub>3</sub> Solution:****Composition** per liter:

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.28g
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Micronutrient Solution:****Composition** per liter:

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.28g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

**Preparation of Micronutrient Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Readjust pH to 8.2. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation of cyanobacteria, including thermophilic species.

**Castenholz Medium****Composition** per liter:

Tryptone .....	1.0g
Yeast extract .....	1.0g
NaNO <sub>3</sub> .....	689.0mg
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	140.0mg
KNO <sub>3</sub> .....	103.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	100.0mg
Nitrilotriacetic acid .....	100.0mg
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	60.0mg
NaCl .....	8.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.2mg
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.47mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	46.0µg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	25.04µg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	25.0µg

pH 8.2 ± 0.2 at 25°C

**Preparation of Medium:** Combine components. Mix thoroughly. Adjust pH to 8.2 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thermus aquaticus*.

### **Castenholz ND Medium (Medium ND)**

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	0.11g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.06g
NaCl .....	8.0mg
FeCl <sub>3</sub> solution .....	1.0mL
Micronutrient solution .....	0.5mL

pH 7.5 ± 0.2 at 25°C

**FeCl<sub>3</sub> Solution:****Composition** per liter:

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.28g
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Micronutrient Solution:****Composition** per liter:

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.28g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

**Preparation of Micronutrient Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Readjust pH to 8.2. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation of cyanobacteria, including thermophilic species, that require reduced nitrogen concentrations.

### Castenholz TYE Medium (Castenholz Trypticase Yeast Extract Medium)

#### Composition per liter:

Castenholz salts, 2×	500.0mL
1% TYE	100.0mL

pH 7.6 ± 0.2 at 25°C

#### Castenholz Salts, 2×

##### Composition per liter:

Agar	30.0g
NaNO <sub>3</sub>	1.4g
Na <sub>2</sub> HPO <sub>4</sub>	0.22g
KNO <sub>3</sub>	0.21g
Nitrilotriacetic acid	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
CaSO <sub>4</sub> ·2H <sub>2</sub> O	0.12g
NaCl	0.016g
FeCl <sub>3</sub> (0.03% solution)	2.0mL
Nitsch's trace elements	2.0mL

**Preparation of Castenholz Salts, 2×** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 8.2. Autoclave for 15 min at 15 psi pressure–121°C.

#### Nitsch's Trace Elements:

##### Composition per liter:

MnSO <sub>4</sub>	2.2g
H <sub>3</sub> BO <sub>3</sub>	0.5g
ZnSO <sub>4</sub>	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.046g
Na <sub>2</sub> MoO <sub>4</sub>	0.025g
CuSO <sub>4</sub>	0.016g
H <sub>2</sub> SO <sub>4</sub>	0.5mL

**Preparation of Nitsch's Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### 1% TYE:

##### Composition per liter:

Pancreatic digest of casein	10.0g
Yeast extract	10.0g

**Preparation of 1% TYE:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 500.0mL of sterile Castenholz salts, 2×, 100.0mL of sterile 1% TYE, and 400.0mL of sterile distilled/deionized water. Adjust pH to 7.6.

**Use:** For the cultivation and maintenance of *Thermonema lapsum* and *Thermus* species.

### Castenholz TYE Medium with 2% Trypticase Yeast Extract

#### Composition per liter:

Castenholz salts, 2×	500.0mL
2% TYE	100.0mL

pH 7.6 ± 0.2 at 25°C

#### Castenholz Salts, 2×

##### Composition per liter:

Agar	30.0g
NaNO <sub>3</sub>	1.4g
Na <sub>2</sub> HPO <sub>4</sub>	0.22g
KNO <sub>3</sub>	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Nitrilotriacetic acid	0.2g
CaSO <sub>4</sub> ·2H <sub>2</sub> O	0.12g

NaCl	0.016g
FeCl <sub>3</sub> solution (0.03% solution)	2.0mL
Nitsch's trace elements	2.0mL

**Preparation of Castenholz Salts, 2×** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 8.2. Autoclave for 15 min at 15 psi pressure–121°C.

#### Nitsch's Trace Elements:

##### Composition per liter:

MnSO <sub>4</sub>	2.2g
H <sub>3</sub> BO <sub>3</sub>	0.5g
ZnSO <sub>4</sub>	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.046g
Na <sub>2</sub> MoO <sub>4</sub>	0.025g
CuSO <sub>4</sub>	0.016g
H <sub>2</sub> SO <sub>4</sub>	0.5mL

**Preparation of Nitsch's Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### 2% TYE:

##### Composition per liter:

Pancreatic digest of casein	20.0g
Yeast extract	20.0g

**Preparation of 2% TYE:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 500.0mL of sterile Castenholz salts, 2×, 100.0mL of sterile 2% TYE, and 400.0mL of sterile distilled/deionized water. Adjust pH to 7.6.

**Use:** For the cultivation and maintenance of *Thermus* species.

### Caulobacter Medium

#### Composition per liter:

Agar	10.0g
Peptone	2.0g
Yeast extract	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Riboflavin	1.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Caulobacter* species from fresh water.

### Caulobacter Medium

#### Composition per liter:

Agar	10.0g
Peptone	0.5g
Seawater, filtered	1.0L

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Caulobacter* species from marine isolates.

**Caulobacter Medium****Composition per liter:**

Glucose .....	1.0g
Peptone .....	1.0g
Yeast extract .....	1.0g
Salt solution .....	100.0mL

**Salt Solution:****Composition per 100.0mL:**

EDTA .....	0.1g
KNO <sub>3</sub> .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.066g
MgSO <sub>4</sub> .....	0.033g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.3mg
NaBO <sub>3</sub> ·4H <sub>2</sub> O .....	2.63mg
MgCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81mg
CaCl <sub>2</sub> .....	1.2mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·7H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·H <sub>2</sub> O .....	0.02mg

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enrichment of *Stella* species from polluted waters.

**Caulobacter Medium****Composition per liter:**

Agar .....	10.0g
Peptone .....	2.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Asticcacaulis excentricus*, *Caulobacter* species, *Labrys monachus*, *Pedomicrobium* species, *Pirellula staleyi*, *Pseudomonas carboxydohydrogena*, and *Stella* species.

**Caulobacter Medium II****Composition per liter:**

Peptone .....	10.0g
Yeast extract .....	3.0g
Seawater .....	1.0L

pH 7.2–7.4 at 25°C

**Preparation of Medium:** Add components to filtered aged seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2–7.4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Caulobacter halobacteroides* and *Caulobacter maris*.

**Caulobacter Medium with Riboflavin****Composition per liter:**

Peptone .....	10.0g
Yeast extract .....	3.0g

Riboflavin .....	1.0mg
Seawater .....	1.0L

pH 7.2–7.4 at 25°C

**Preparation of Medium:** Add components to filtered aged seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Caulobacter vibrioides*.

**CCVC Medium**  
**(Cephalothin Cycloheximide**  
**Vancomycin Colistin Medium)**

**Composition per liter:**

BCYE-alpha base .....	990.0mL
Antibiotic supplement .....	10.0mL

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**BCYE-Alpha Base:****Composition per liter:**

Agar .....	15.0g
Yeast extract .....	10.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)-amino]-ethane sulfonic acid) .....	10.0g
Charcoal, activated .....	2.0g
α-Ketoglutarate .....	1.0g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.25g
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL

**L-Cysteine·HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.4g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of BCYE-Alpha Base:** Add components, except L-cysteine·HCl·H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boiling for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 4.0mL of L-cysteine·HCl·H<sub>2</sub>O solution. Mix thoroughly.

**Antibiotic Supplement Solution:****Composition per 10.0mL:**

Cycloheximide .....	80.0mg
Colistin .....	16.0mg
Cephalothin .....	4.0mg
Vancomycin .....	0.5mg

**Preparation of Antibiotic Supplement Solution:** Add components to 10.0mL of distilled/deionized water. Filter sterilize.

**Preparation of Medium:** To cooled BCYE-alpha base, add 10.0mL sterile antibiotic supplement. Mix thoroughly. Adjust pH to 6.9 with sterile 1N KOH. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the selective isolation and cultivation of *Legionella* species from environmental samples.

**Cellulolytic Agar with Sea Salts****Composition per liter:**

Agar .....	20.0g
NH <sub>4</sub> Cl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.65g

Yeast extract.....	1.2g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.5g
Cellulose suspension.....	200.0mL
Filtered seawater.....	200.0mL
Mineral solution.....	150.0mL
Resazurin (0.1% solution).....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Cellulose Suspension:****Composition** per 200.0mL:

Cellulose powder, Whatman CF11.....	8.0g
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**Preparation of Cellulose Suspension:** Add cellulose powder to 200.0mL of distilled/deionized water and mix thoroughly.

**Mineral Solution:****Composition** per liter:

NaCl.....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
CaCl <sub>2</sub> .....	0.6g
MgSO <sub>4</sub> .....	0.6g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium anaerobically under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2 with 5M NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Clostridium papyrosolvens* and other marine bacteria that can utilize cellulose as a carbon source.

**Cellulolytic Agar for Thermophiles****Composition** per liter:

Agar.....	30.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.65g
NH <sub>4</sub> SO <sub>4</sub> .....	1.6g
Yeast extract.....	1.0g
NaCl.....	0.96g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> .....	0.096g
MgSO <sub>4</sub> .....	0.096g
Cellulose suspension.....	200.0mL
Resazurin (0.1% solution).....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Cellulose Suspension:****Composition** per 200.0mL:

Cellulose powder, Whatman CF11.....	8.0g
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**Preparation of Cellulose Suspension:** Add cellulose powder to 200.0mL of distilled/deionized water and mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium anaerobically in 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2 with 5M NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For cultivation of *Clostridium stercorarium* and other bacteria that can utilize cellulose as a carbon source.

**Cellulolytic Broth with Sea Salts****Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	1.65g
NH <sub>4</sub> Cl.....	1.0g
Yeast extract.....	0.6g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.5g

Filtered seawater.....	200.0mL
Mineral solution.....	150.0mL
Resazurin (0.1% solution).....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Mineral Solution:****Composition** per liter:

NaCl.....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
CaCl <sub>2</sub> .....	0.6g
MgSO <sub>4</sub> .....	0.6g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium anaerobically in 100% N<sub>2</sub> atmosphere. Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.2 with 5M NaOH. Distribute into tubes or flasks that contain cellulose as a strip (4.5cm × 1.0cm) of Whatman No. 1 filter paper. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Clostridium papyrosolvens* and other marine bacteria that can utilize cellulose as a carbon source.

**Cellulolytic Medium****Composition** per liter:

NaHCO <sub>3</sub> .....	2.06g
Cellulose.....	2.0g
NH <sub>4</sub> Cl.....	0.68g
K <sub>2</sub> HPO <sub>4</sub> .....	0.296g
KH <sub>2</sub> PO <sub>4</sub> .....	0.18g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	61.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	21.0mg
Nitritotriacetic acid.....	15.0mg
NaCl.....	10.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	5.0mg
CoCl <sub>2</sub> ·H <sub>2</sub> O.....	1.0mg
Resazurin.....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.1mg
H <sub>3</sub> BO <sub>3</sub> .....	0.1mg
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1mg
Wolfe's vitamin solution.....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution.....	5.0mL
pH 7.5 ± 0.2 at 25°C	

**Wolfe's Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
Calcium DL-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:****Composition** per 20.0mL:L-Cysteine-HCl-H<sub>2</sub>O ..... 1.0g

**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Wolfe's vitamin solution and L-cysteine-HCl-H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile Wolfe's vitamin solution and 5.0mL of sterile L-cysteine-HCl-H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of cellulolytic bacteria.

**Cellulolytic Medium with  
Rumen Fluid and Soluble Starch**

**Composition** per liter:

Basal medium ..... 975.0mL

Alkaline solution ..... 25.0mL

pH 6.8 ± 0.2 at 25°C

**Basal Medium:****Composition** per 975.0mL:

Agar ..... 15.0g

NaHCO<sub>3</sub> ..... 6.37g

Pancreatic digest of casein ..... 5.0g

Cellobiose ..... 5.0g

Soluble starch ..... 5.0g

NaCl ..... 0.9g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.9gK<sub>2</sub>HPO<sub>4</sub> ..... 0.45gKH<sub>2</sub>PO<sub>4</sub> ..... 0.45gMgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18gCaCl<sub>2</sub> ..... 0.09g

Resazurin ..... 1.0mg

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Gently heat and bring to boiling under a gas phase of 98% CO<sub>2</sub> + 2% H<sub>2</sub>. Cool slightly.

**Alkaline Solution:****Composition** per 25.0mL:L-Cysteine-HCl-H<sub>2</sub>O ..... 0.25gNa<sub>2</sub>S·9H<sub>2</sub>O ..... 0.25g

**Preparation of Alkaline Solution:** Add components to 25.0mL of distilled/deionized water. Mix thoroughly. Prepare freshly.

**Preparation of Medium:** Prepare 975.0mL of basal medium. Heat to boiling and cool to 25°C. Add 25.0mL of freshly prepared alkaline solution. Distribute into tubes using anaerobic techniques under a gas phase of 98% CO<sub>2</sub> + 2% H<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 6.8.

**Use:** For the cultivation of *Selenomonas ruminantium* and *Succinimonas amylolytica*.

***Cellulomonas fermentans* Medium**

**Composition** per liter:

Yeast extract ..... 5.0g

K<sub>2</sub>HPO<sub>4</sub> ..... 2.21gKH<sub>2</sub>PO<sub>4</sub> ..... 1.5g(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.3gMgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1gCaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.02gFeSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.25mg

Resazurin ..... 1.0mg

Cellobiose solution ..... 50.0mL

NaHCO<sub>3</sub> solution ..... 10.0mL

L-Cysteine-HCl solution ..... 10.0mL

pH 7.4 ± 0.2 at 25°C

**Cellobiose Solution:****Composition** per 50.0mL:

D-Cellobiose (or cellulose) ..... 5.0g

**Preparation of Cellobiose Solution:** Add cellobiose (or cellulose) to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition** per 10.0mL:NaHCO<sub>3</sub> ..... 0.8g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**L-Cysteine-HCl Solution:****Composition** per 10.0mL:

L-Cysteine-HCl ..... 0.5g

**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except cellobiose solution, NaHCO<sub>3</sub> solution, and L-cysteine-HCl solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> for 10 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile cellobiose solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, and 10.0mL of sterile L-cysteine-HCl solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Cellulomonas fermentans*.

***Cellulomonas* PTYG Medium  
(*Cellulomonas* Peptone Tryptone  
Yeast Extract Glucose Medium)**

**Composition** per liter:

Agar ..... 15.0g

Glucose ..... 5.0g

Peptone ..... 5.0g

Pancreatic digest of casein ..... 5.0g

Yeast extract ..... 5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Cellulomonas* species.

**Cellulose Anaerobe Medium  
(LMG Medium 94)**

**Composition** per liter:NaHCO<sub>3</sub> ..... 2.1g

Cellulose ..... 2.0g

NH<sub>4</sub>Cl ..... 0.68gKH<sub>2</sub>PO<sub>4</sub> ..... 0.18g

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.12g
K <sub>2</sub> HPO <sub>4</sub> .....	296.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	61.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	21.0mg
Nitritotriacetic acid.....	15.0mg
NaCl.....	10.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	5.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
Resazurin .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.1mg
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.1mg
H <sub>3</sub> BO <sub>3</sub> .....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
Vitamin solution.....	5.0mL
Cysteine hydrochloride solution.....	5.0mL

pH 7.1 ± 0.2 at 25°C

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**L-Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O.....	0.5g
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**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components except vitamin solution and cysteine hydrochloride solution. Add 485.0mL distilled/deionized water. Adjust pH to 7.1. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL of sterile vitamin solution and 5.0mL sterile cysteine hydrochloride solution. Mix thoroughly. Aseptically distribute to tubes or flasks.

**Use:** For the cultivation of cellulose-utilizing anaerobic bacteria.

**Cellulose Broth****Composition per liter:**

Cellulose, powdered.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeCl <sub>3</sub> .....	0.02g

pH 7.0–7.5 at 25°C

**Preparation of Medium:** Add cellulose to 100.0mL of distilled/deionized water. Mix thoroughly. In a separate flask, add remaining components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Auto-

clave both solutions separately for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine the two sterile solutions. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

**Cellulose Overlay Agar****Composition per plate:**

Stan 5 agar.....	15.0mL
Cellulose overlay agar.....	5.0mL

**Stan 5 Agar:****Composition per liter:**

Solution B.....	650.0mL
Solution A.....	350.0mL

**Solution A:****Composition per 350.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
Trace elements solution.....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 350.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution:****Composition per liter:**

EDTA.....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> .....	0.02g
KBr.....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
BaCl <sub>2</sub> .....	5.0mg
LiCl.....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O.....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Solution B:****Composition per 650.0mL:**

Agar.....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 650.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Stan 5 Agar:** Aseptically combine 350.0mL of cooled, sterile solution A and 650.0mL of cooled, sterile solution B. Mix thoroughly.

**Cellulose Overlay Agar:****Composition per liter:**

Solution A.....	350.0mL
Solution B.....	650.0mL

**Solution A:****Composition per 350.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
Trace elements solution.....	1.0mL



**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 350.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Trace Elements Solution:

##### Composition per liter:

EDTA .....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> .....	0.02g
KBr .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
BaCl <sub>2</sub> .....	5.0mg
LiCl .....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution B:

##### Composition per 650.0mL:

Agar .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 650.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Cellulose Overlay Agar:** Aseptically combine 350.0mL of cooled, sterile solution A and 650.0mL of cooled, sterile solution B. Mix thoroughly.

**Preparation of Medium:** Pour cooled, sterile Stan 5 agar into sterile Petri dishes in 15.0mL volumes. Allow agar to solidify. Overlay each plate with 5.0mL of cellulose overlay agar.

**Use:** For the cultivation of myxobacteria.

#### *Cellvibrio* Medium

##### Composition per liter:

Agar .....	15.0g
NaNO <sub>3</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Cellvibrio mixtus*, *Cytophaga aurantiaca*, *Cytophaga hutchinsonii*, and *Sporocytophaga myxococcoides*.

#### CENS Medium (DSMZ Medium 748)

##### Composition per liter:

Soytone .....	4.0g
NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	75.0mg
EDTA .....	5.0mg
Vitamin B <sub>12</sub> .....	20.0μg
Biotin .....	15.0μg
Na-pyruvate solution .....	10.0mL
Chelated iron solution .....	2.0mL
True Blue Trace elements .....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Na-pyruvate Solution:

##### Composition per 10.0mL:

Na-pyruvate .....	2.2g
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**Preparation of Na-pyruvate Solution:** Add Na-pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### True Blue Trace Elements:

##### Composition per 250mL:

EDTA .....	2.5g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2g
H <sub>3</sub> BO <sub>3</sub> .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	50.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	50.0mg
CoCl <sub>2</sub> ·2H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	5.0mg
NaVO <sub>3</sub> ·H <sub>2</sub> O .....	5.0mg

**Preparation of True Blue Trace Elements:** Add components one at a time to approximately 200.0mL distilled/deionized water. Bring final volume to 250.0mL with additional distilled/deionized water.

#### Chelated Iron Solution:

##### Composition per 900mL:

EDTA .....	2.0g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0g
HCl .....	3.0mL

**Preparation of Chelated Iron Solution:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except Na-pyruvate solution, to distilled/deionized water and bring volume to 990.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL sterile Na-pyruvate solution. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Rhodocista centenaria*=*Rhodospirillum centenum*.

#### Centenum Medium

##### Composition per liter of tap water:

Agar .....	20.0g
Yeast extract .....	10.0g
Sodium pyruvate .....	2.2g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
Vitamin B <sub>12</sub> .....	0.02mg

pH 7.0–7.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Rhodospirillum* species.

#### Cetrimide Agar, Non-USP

##### Composition per liter:

Beef heart, solids from infusion	500.0g
Agar	15.0g
Tryptose	10.0g
NaCl	5.0g
Cetrimide	0.9g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 13 psi pressure–118°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation, cultivation, and identification of *Pseudomonas aeruginosa* and other Gram-negative, nonfermentative bacteria.

#### Cetrimide Agar, USP (Pseudosel® Agar)

##### Composition per liter:

Pancreatic digest of gelatin	20.0g
Agar	13.6g
K <sub>2</sub> SO <sub>4</sub>	10.0g
MgCl <sub>2</sub>	1.4g
Cetrimide	0.3g
Glycerol	10.0mL

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 13 psi pressure–118°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation, cultivation, and identification of *Pseudomonas aeruginosa* and other Gram-negative, nonfermentative bacteria.

#### CHCA Salts Medium (Cyclohexane Carboxylic Acid Salts Medium)

##### Composition per liter:

K <sub>2</sub> HPO <sub>4</sub>	3.5g
KH <sub>2</sub> PO <sub>4</sub>	1.5g
Cyclohexane carboxylic acid	1.0g
NH <sub>4</sub> NO <sub>3</sub>	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
Yeast extract	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of bacteria that can utilize cyclohexane carboxylic acid as a carbon source. For the cultivation and maintenance of *Arthrobacter globiformis*.

#### Chitin Agar

##### Composition per liter:

Agar	15.0g
Chitin, precipitated	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2.0g
Na <sub>2</sub> HPO <sub>4</sub>	1.1g
KH <sub>2</sub> PO <sub>4</sub>	0.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
FeSO <sub>4</sub>	1.0mg
MnSO <sub>4</sub>	1.0mg

##### Chitin, Precipitated:

##### Composition per 2.5L:

Chitin	40.0g
HCl, concentrated	400.0mL

**Preparation of Chitin, Precipitated:** Add chitin to 400.0mL of cold concentrated HCl. Add this solution to 2.0L of distilled/deionized water at 5°C. Filter the solution through Whatman #1 filter paper. Dialyze the precipitated chitin against tap water for 12 hr. Adjust the pH to 7.0 with KOH.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

#### Chitin Agar

##### Composition per liter:

Agar	20.0g
Chitin	4.0g
K <sub>2</sub> HPO <sub>4</sub>	0.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.3g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.001g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.001g

pH 8.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation and cultivation of streptomycetes.

#### Chlamydomonas Enriched Medium

##### Composition per liter:

Agar	15.0g
Sodium acetate, anhydrous	2.0g
Tryptose	2.0g
Yeast extract	2.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Chlamydomonas reinhardtii*.

#### Chlorinated Fatty Acid Medium

##### Composition per 1001.0mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.5g
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MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.075g
2-Chloropropionate .....	0.54g
Phosphate buffer (20mM solution, pH 7.2) .....	1.0L
Trace elements solution .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Trace Elements Solution:****Composition per liter:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.0g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.25g
H <sub>3</sub> BO <sub>3</sub> .....	0.1g
NH <sub>4</sub> VO <sub>3</sub> .....	0.1g
NiSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Alcaligenes* species.

**Chlorobiaceae Medium 1****Composition per 4990.0mL:**

Solution 1 .....	4.0L
O <sub>2</sub> -free water .....	860.0mL
NaHCO <sub>3</sub> solution .....	100.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
Trace elements solution .....	5.0mL
Vitamin B <sub>12</sub> solution .....	5.0mL
pH 6.8 ± 0.2 at 25°C	

**Solution 1:****Composition per 4.0L:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.5g
KCl .....	1.7g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C. Cool to 25°C under 100% N<sub>2</sub>. Saturate with CO<sub>2</sub> by stirring under 100% CO<sub>2</sub> for 30 min.

**O<sub>2</sub>-Free Water:****Composition per 860.0mL:**

H <sub>2</sub> O .....	860.0mL
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**Preparation of O<sub>2</sub>-Free Water:** Autoclave H<sub>2</sub>O for 15 min at 15 psi pressure–121°C. Cool to 25°C under 100% N<sub>2</sub>.

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gas with 100% CO<sub>2</sub> for 20 min. Filter sterilize with positive CO<sub>2</sub> pressure.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water. Mix thoroughly. Gas with 100% N<sub>2</sub> for 15 min in a screw-capped bottle. Tightly close cap. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Trace Elements Solution:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
HCl (25% solution) .....	6.5mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Vitamin B<sub>12</sub> Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	2.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 4.0L of sterile, CO<sub>2</sub>-saturated solution 1, aseptically add the remaining components. Mix thoroughly. Adjust pH to 6.8. Aseptically distribute into sterile 100.0mL bottles using positive pressure of 95% N<sub>2</sub> + 5% CO<sub>2</sub>. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the isolation and cultivation of members of the Chlorobiaceae.

**Chlorobiaceae Medium 2****Composition per 1051.0mL:**

Solution 1 .....	950.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	60.0mL
NaHCO <sub>3</sub> solution .....	40.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL
pH 6.8 ± 0.2 at 25°C	

**Solution 1:****Composition per 950.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements solution SL-8 .....	1.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution SL-8:****Composition per liter:**

Sodium EDTA .....	5.2g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 5.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B<sub>12</sub> ..... 2.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 950.0mL of cooled, sterile solution 1, aseptically add 60.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 40.0mL of sterile NaHCO<sub>3</sub> solution, and 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Adjust pH to 6.8 with sterile H<sub>2</sub>SO<sub>4</sub> or Na<sub>2</sub>CO<sub>3</sub>. Aseptically distribute into sterile 50.0mL or 100.0mL bottles with metal screw-caps and rubber seals. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the isolation and cultivation of freshwater and soil members of the Chlorobiaceae.

***Chlorobium thiosulfatophilum* Medium**

**Composition** per 1050.0mL:

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
NH<sub>4</sub>Cl ..... 1.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.5g  
Solution A ..... 20.0mL  
Solution B ..... 20.0mL  
Solution C ..... 10.0mL  
Trace elements solution ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:**

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 10.0g

**Preparation of Solution A:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:**

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 10.0g

**Preparation of Solution B:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:**

**Composition** per 100.0mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·9H<sub>2</sub>O ..... 10.0g

**Preparation of Solution C:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:**

**Composition** per liter:

FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 2.7g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.1g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 50.0mg  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 5.0mg  
MnCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except solution A, solution B, and solution C, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 7.0–7.2 with H<sub>3</sub>PO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.2mL of sterile solution A, 0.2mL of sterile solution B, and 0.1mL of sterile solution C for each 10.0mL of medium. Mix thoroughly. Use immediately.

**Use:** For the cultivation and maintenance of *Chlorobium limnicola*.

**Chlorobutane Medium**

**Composition** per 1002.0mL:

NH<sub>4</sub>NO<sub>3</sub> ..... 4.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.5g  
Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O ..... 1.5g  
CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 10.0mg  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 10.0mg  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 5.0mg  
Yeast extract ..... 5.0mg  
1-Chlorobutane ..... 2.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of 1-Chlorobutane:** Filter sterilize.

**Preparation of Medium:** Add components, except 1-chlorobutane, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 2.0mL of sterile 1-chlorobutane. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Corynebacterium* species.

***Chloroflexus* Agar**

**Composition** per liter:

Agar ..... 15.0g  
Glycyl-glycine ..... 0.5g  
Yeast extract ..... 0.5g  
Na<sub>2</sub>S ..... 0.5g  
NH<sub>4</sub>Cl ..... 0.2g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
Nitrilotriacetic acid ..... 0.1g  
NaNO<sub>3</sub> ..... 0.689g  
Na<sub>2</sub>HPO<sub>4</sub> ..... 0.111g  
KNO<sub>3</sub> ..... 0.103g  
CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.06g  
NaCl ..... 8.0mg  
FeCl<sub>3</sub> solution ..... 1.0mL  
Micronutrient solution ..... 1.0mL

pH 8.2–8.4 at 25°C

**FeCl<sub>3</sub> Solution:**

**Composition** per liter:

FeCl<sub>3</sub> ..... 0.29g

**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Micronutrient Solution:****Composition** per liter:

MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.28g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.045g
CuSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
H <sub>2</sub> SO <sub>4</sub> , concentrated .....	0.5mL

**Preparation of Micronutrient Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.2–8.4. Add Na<sub>2</sub>S. Re-adjust pH to 8.2–8.4. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Chloroflexus aurantiacus*.

***Chloroflexus aggregans* Medium  
(DSMZ Medium 87a)**

**Composition** per 1061.0mL:

Yeast extract .....	1.0g
Glycyl-glycine .....	1.0g
NaNO <sub>3</sub> .....	0.5g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
KNO <sub>3</sub> .....	0.1g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Neutralized sulfide solution .....	11.0mL
Ferric citrate solution .....	5.0mL
Trace elements solution SL-6 .....	1.0mL
pH 8.2 ± 0.2 at 25°C	

**Ferric Citrate Solution:****Composition** per 100.0mL:

Ferric citrate .....	0.1mg
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min.

**Trace Elements Solution SL-6:****Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Neutralized Sulfide Solution:****Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.5g
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**Preparation of Neutralized Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

Adjust pH to about 7.3 with sterile 2M H<sub>2</sub>SO<sub>4</sub>. Do not open the bottle to add H<sub>2</sub>SO<sub>4</sub>; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add components, except neutralized sulfide solution, to distilled/deionized water and bring volume to 1050.0mL. Mix thoroughly. Adjust pH to 8.2. Gently heat and bring to boiling. Continue boiling for 3–4 min under 100% N<sub>2</sub>. Distribute 90.0mL of medium into 100mL screw-capped bottles with rubber septa under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Using a sterile syringe, inject 1.0mL of neutralized sulfide solution into each bottle. Incubate the culture at 50°C at a light intensity of 300–500 lux. For heavy cell suspension supplement periodically with sterile yeast extract solution to yield a final concentration of 0.1%.

**Use:** For the growth and maintenance of *Chloroflexus aggregans* and *Roseiflexus castenholzii*.

***Chloroflexus* Medium, Modified**

**Composition** per 1001.0 mL:

Glycyl-glycine .....	1.0g
Yeast extract .....	1.0g
NaNO <sub>3</sub> .....	0.5g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Neutralized sulfide solution .....	11.0mL
Ferric citrate solution .....	1.0mL
Trace elements solution SL-6 .....	1.0mL
pH 8.2 ± 0.2 at 25°C	

**Neutralized Sulfide Solution:****Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.5g
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**Preparation of Neutralized Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.3 with sterile 2M H<sub>2</sub>SO<sub>4</sub>. Do not open the bottle to add H<sub>2</sub>SO<sub>4</sub>; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Ferric Citrate Solution:****Composition** per 100.0mL:

Ferric citrate .....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Trace Elements Solution SL-6:****Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except neutralized sulfide solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min under 100% N<sub>2</sub>. Distribute 90.0mL of medium into 100mL screw-capped bottles under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Using a sterile syringe, inject 1.0mL of neutralized sulfide solution into each bottle.

**Use:** For the growth and maintenance of *Chloroflexus aurantiacus*.

#### Chlorohydroxybenzoic Acid Medium

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	4.25g
NH <sub>4</sub> Cl	2.0g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	1.0g
5-Chloro-2-hydroxybenzoic acid	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Nitrilotriacetic acid	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.012g
MnSO <sub>4</sub> ·H <sub>2</sub> O	3.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	3.0mg
CoSO <sub>4</sub>	1.0mg

pH 7.0–7.4 at 25°C

**Preparation of Medium:** Add 5-chloro-2-hydroxybenzoic acid to 800.0mL of distilled/deionized water. Adjust pH to 7.0 with NaOH. Add remaining components and bring volume to 1.0L. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of bacteria that can utilize 5-chloro-hydroxybenzoic acid. For the cultivation of ATCC strain 35944.

#### Cholera Medium TCBS

**Composition per liter:**

Sucrose	20.0g
Agar	14.0g
Peptone	10.0g
NaCl	10.0g
Sodium citrate	10.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O	10.0g
Ox bile	8.0g
Yeast extract	5.0g
Ferric citrate	1.0g
Bromothymol Blue	0.04g
Thymol Blue	0.04g

pH 8.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Pour into sterile Petri dishes. Dry agar plates before using.

**Use:** For the growth of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and other *Vibrio* species.

#### Chopped Meat Carbohydrate Medium with Rumen Fluid

**Composition per 1390.0mL:**

Peptone	30.0g
K <sub>2</sub> HPO <sub>4</sub>	5.0g
Yeast extract	5.0g
Glucose	4.0g
Cellobiose	1.0g

Maltose	1.0g
Starch	1.0g
L-Cysteine·HCl·H <sub>2</sub> O	0.5g
Chopped meat extract filtrate	1.0L
Chopped meat extract solids	200.0mL
Rumen fluid	150.0mL
Resazurin (0.025% solution)	4.0mL

pH 7.0 ± 0.2 at 25°C

#### Chopped Meat Extract:

**Composition per liter:**

Beef or horse meat	500.0g
NaOH (1N solution)	25.0mL

**Preparation of Chopped Meat Extract:** Use lean beef or horse meat. Remove fat and connective tissue. Grind. Add meat and NaOH to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling while stirring. Cool to 25°C. Remove fat from surface. Filter. Reserve ground meat particles and filtrate. Add distilled/deionized water to filtrate and bring volume to 1.0L.

**Preparation of Medium:** To 1.0L of chopped meat extract filtrate, add the remaining components, except L-cysteine·HCl·H<sub>2</sub>O and chopped meat solids. Mix thoroughly. Gently heat to boiling. Cool to room temperature. Add the L-cysteine·HCl·H<sub>2</sub>O. Adjust pH to 7.0. Distribute 1 part chopped meat solids (by volume) and 5 parts of liquid (by volume) into tubes under O<sub>2</sub>-free 97% N<sub>2</sub> + 3% H<sub>2</sub>. Cap with rubber stoppers and place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation of anaerobic bacteria, including *Fusobacterium prausnitzii*, *Eubacterium* species, and *Prevotella ruminicola*.

#### Chopped Meat Carbohydrate Medium with Rumen Fluid (ATCC Medium 1016)

**Composition per 1390.0mL:**

Peptone	30.0g
K <sub>2</sub> HPO <sub>4</sub>	5.0g
Yeast extract	5.0g
Cellobiose	1.0g
Maltose	1.0g
Starch	1.0g
L-Cysteine·HCl·H <sub>2</sub> O	0.5g
Chopped meat extract filtrate	1.0L
Chopped meat extract solids	200.0mL
Rumen fluid	150.0mL
Resazurin (0.025% solution)	4.0mL

pH 7.0 ± 0.2 at 25°C

#### Chopped Meat Extract:

**Composition per liter:**

Beef or horse meat	500.0g
NaOH (1N solution)	25.0mL

**Preparation of Chopped Meat Extract:** Use lean beef or horse meat. Remove fat and connective tissue. Grind. Add meat and NaOH to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling while stirring. Cool to 25°C. Remove fat from surface. Filter. Reserve ground meat particles and filtrate. Add distilled/deionized water to filtrate and bring volume to 1.0L.

**Preparation of Medium:** To 1.0L of chopped meat extract filtrate, add the remaining components, except L-cysteine·HCl·H<sub>2</sub>O and chopped meat solids. Mix thoroughly. Gently heat to boiling. Cool to room temperature. Add the L-cysteine·HCl·H<sub>2</sub>O. Adjust pH to 7.0. Distribute 1 part chopped meat solids (by volume) and 5 parts of liquid (by

volume) into tubes under O<sub>2</sub>-free 97% N<sub>2</sub> + 3% H<sub>2</sub>. Cap with rubber stoppers and place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation of anaerobic bacteria, including *Butyrivibrio crossotus*, *Eubacterium* species, and *Ruminococcus* species.

### CHROMagar *E. coli*

**Composition** per liter:  
Proprietary.

**Source:** CHROMagar *E. coli* is available from CHROMagar Microbiology.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat in a boiling water bath or steam bath. Shake periodically during heating to dissolve components. Heat long enough with shaking every 5 min to ensure complete dissolution. Do not overheat. Adding tellurite can increase specificity. Cool to 45–50°C. Pour into sterile Petri dishes.

**Use:** For the differentiation and presumptive identification of *Escherichia coli* which forms blue colonies.

### CHROMagar ECC

**Composition** per liter:  
Proprietary.

**Source:** CHROMagar EEC is available from CHROMagar Microbiology.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat in a boiling water bath or steam bath. Shake periodically during heating to dissolve components. Heat long enough with shaking every 5 min to ensure complete dissolution. Do not overheat. Adding tellurite can increase specificity. Cool to 45–50°C. Pour into sterile Petri dishes.

**Use:** For the differentiation and presumptive identification of *Escherichia coli* and other coliform bacteria which form red colonies.

### CHROMagar O157

**Composition** per liter:  
Proprietary.

**Source:** CHROMagar O157 is available from CHROMagar Microbiology.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat in a boiling water bath or steam bath. Shake periodically during heating to dissolve components. Heat long enough with shaking every 5 min to ensure complete dissolution. Do not overheat. Adding tellurite can increase specificity. Cool to 45–50°C. Pour into sterile Petri dishes.

**Use:** For the differentiation and presumptive identification of *Escherichia coli* O157.

### CHROMagar *Vibrio*

**Composition** per liter:  
Proprietary.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat in a boiling water bath or steam bath. Shake periodically during heating to dissolve components. Heat long enough with shaking every 5 min to ensure complete dissolution. Do not overheat. Adding tellurite can increase specificity. Cool to 45–50°C. Pour into sterile Petri dishes.

**Source:** CHROMagar *Vibrio* is available from CHROMagar Microbiology.

**Use:** For the differentiation and presumptive identification of *Vibrio parahaemolyticus* which form mauve colonies; *Vibrio cholerae* form turquoise blue colonies and *Vibrio alginolyticus* colonies are colorless.

### Chromatiaceae Medium 1

**Composition** per 4990.0mL:

Solution 1 .....	4.0L
O <sub>2</sub> -free water .....	860.0mL
NaHCO <sub>3</sub> solution .....	100.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
Trace elements solution .....	5.0mL
Vitamin B <sub>12</sub> solution .....	5.0mL
pH 7.3 ± 0.2 at 25°C	

#### Solution 1:

**Composition** per 4.0L:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.5g
KCl .....	1.7g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C. Cool to 25°C under 100% N<sub>2</sub>. Saturate with CO<sub>2</sub> by stirring under 100% CO<sub>2</sub> for 30 min.

#### O<sub>2</sub>-Free Water:

**Composition** per 860.0mL:

H <sub>2</sub> O .....	860.0mL
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**Preparation of O<sub>2</sub>-Free Water:** Autoclave H<sub>2</sub>O for 15 min at 15 psi pressure–121°C. Cool to 25°C under 100% N<sub>2</sub>.

#### NaHCO<sub>3</sub> Solution:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gas with 100% CO<sub>2</sub> for 20 min. Filter sterilize with positive CO<sub>2</sub> pressure.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water. Mix thoroughly. Gas with 100% N<sub>2</sub> for 15 min in a screw-capped bottle. Tightly close cap. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Trace Elements Solution:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
HCl (25% solution) .....	6.5mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to

1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B<sub>12</sub> .....2.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 4.0L of sterile, CO<sub>2</sub>-saturated solution 1, aseptically add the remaining components. Mix thoroughly. Adjust pH to 7.3. Aseptically distribute into sterile 100.0mL bottles using positive pressure of 95% N<sub>2</sub> + 5% CO<sub>2</sub>. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the isolation and cultivation of members of the Chlorobiaceae.

#### **Chromatiaceae Medium 2**

**Composition** per 1051.0mL:

Solution 1 .....950.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution .....60.0mL  
NaHCO<sub>3</sub> solution .....40.0mL  
Vitamin B<sub>12</sub> solution .....1.0mL  
pH 7.3 ± 0.2 at 25°C

#### **Solution 1:**

**Composition** per 950.0mL:

KH<sub>2</sub>PO<sub>4</sub> .....1.0g  
NH<sub>4</sub>Cl .....0.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O .....0.4g  
CaCl<sub>2</sub>·2H<sub>2</sub>O .....0.05g  
Trace elements solution SL-8 .....1.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **Trace Elements Solution SL-8:**

**Composition** per liter:

Disodium EDTA .....5.2g  
FeCl<sub>2</sub>·4H<sub>2</sub>O .....1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O .....0.19g  
MnCl<sub>2</sub>·4H<sub>2</sub>O .....0.1g  
ZnCl<sub>2</sub> .....0.07g  
H<sub>3</sub>BO<sub>3</sub> .....0.06g  
NaMoO<sub>4</sub>·2H<sub>2</sub>O .....0.04g  
CuCl<sub>2</sub>·2H<sub>2</sub>O .....0.02g  
NiCl<sub>2</sub>·6H<sub>2</sub>O .....0.02g

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O .....5.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO<sub>3</sub> .....5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### **Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B<sub>12</sub> .....2.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 950.0mL of cooled, sterile solution 1, aseptically add 60.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 40.0mL of sterile NaHCO<sub>3</sub> solution, and 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Adjust pH to 7.3 with sterile H<sub>2</sub>SO<sub>4</sub> or Na<sub>2</sub>CO<sub>3</sub>. Aseptically distribute into sterile 50.0mL or 100.0mL bottles with metal screw-caps and rubber seals. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the isolation and cultivation of freshwater and soil members of the Chromatiaceae.

#### **Chromatium Medium (ATCC Medium 1449)**

**Composition** per liter:

KH<sub>2</sub>PO<sub>4</sub> .....0.5g  
NH<sub>4</sub>Cl .....0.4g  
MgSO<sub>4</sub>·7H<sub>2</sub>O .....0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O .....0.05g  
Disodium EDTA .....0.01g  
Trace elements .....1.0mL  
NaHCO<sub>3</sub> solution .....50.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution .....50.0mL  
Sodium pyruvate solution .....50.0mL  
pH 7.0 ± 0.2 at 25°C

#### **Trace Elements:**

**Composition** per liter:

Disodium EDTA .....5.2g  
FeCl<sub>2</sub>·4H<sub>2</sub>O .....1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O .....0.19g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O .....0.188g  
MnCl<sub>2</sub>·4H<sub>2</sub>O .....0.1g  
ZnCl<sub>2</sub> .....0.07g  
VOSO<sub>4</sub>·2H<sub>2</sub>O .....0.03g  
NiCl<sub>2</sub>·6H<sub>2</sub>O .....0.025g  
H<sub>3</sub>BO<sub>3</sub> .....6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O .....2.0mg  
Na<sub>2</sub>SeO<sub>3</sub> .....2.0mg

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **NaHCO<sub>3</sub> Solution:**

**Composition** per 50.0mL:

NaHCO<sub>3</sub> .....2.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Filter sterilize. Use freshly prepared solution.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 50.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O .....1.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 50.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Use freshly prepared solution.

#### **Sodium Pyruvate Solution:**

**Composition** per 50.0mL:

Sodium pyruvate .....0.5g



**Preparation of Sodium Pyruvate Solution:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 50.0mL. Filter sterilize. Use freshly prepared solution. Sodium acetate may be substituted for the sodium pyruvate.

**Preparation of Medium:** Add components, except  $\text{NaHCO}_3$  solution,  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, and sodium pyruvate solution, to distilled deionized water and bring volume to 850.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Add the sterile  $\text{NaHCO}_3$  solution, the sterile  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, and the sterile sodium pyruvate solution, in that order. Adjust the pH to 7.0. Distribute into screw-capped tubes or flasks. Fill to capacity.

**Use:** For the cultivation and maintenance of *Chromatium* species.

#### *Chromatium* Medium with NaCl

**Composition** per 1051.0mL:

Solution 1 .....	950.0mL
$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ solution .....	60.0mL
$\text{NaHCO}_3$ solution .....	40.0mL
Vitamin $\text{B}_{12}$ solution .....	1.0mL
pH 3–4 at 25°C	

#### **Solution 1:**

**Composition** per 950.0mL:

$\text{NaCl}$ .....	30.0g
$\text{KH}_2\text{PO}_4$ .....	1.0g
$\text{NH}_4\text{Cl}$ .....	0.5g
$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.4g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.05g
Trace elements solution SL-8 .....	1.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **Trace Elements Solution SL-8:**

**Composition** per liter:

Disodium EDTA .....	5.2g
$\text{FeCl}_2\cdot 4\text{H}_2\text{O}$ .....	1.5g
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.19g
$\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ .....	0.10g
$\text{ZnCl}_2$ .....	0.07g
$\text{H}_3\text{BO}_3$ .....	0.06g
$\text{NaMoO}_4\cdot 2\text{H}_2\text{O}$ .....	0.04g
$\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.02g
$\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.02g

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ Solution:**

**Composition** per 100.0mL:

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ .....	5.0g
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**Preparation of  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **$\text{NaHCO}_3$ Solution:**

**Composition** per 100.0mL:

$\text{NaHCO}_3$ .....	5.0g
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**Preparation of  $\text{NaHCO}_3$  Solution:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### **Vitamin $\text{B}_{12}$ Solution:**

**Composition** per 100.0mL:

Vitamin $\text{B}_{12}$ .....	2.0mg
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**Preparation of Vitamin  $\text{B}_{12}$  Solution:** Add Vitamin  $\text{B}_{12}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 950.0mL of cooled, sterile solution 1, aseptically add 60.0mL of sterile  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, 40.0mL of sterile  $\text{NaHCO}_3$  solution, and 1.0mL of sterile Vitamin  $\text{B}_{12}$  solution. Mix thoroughly. Adjust pH to 7.3 with sterile  $\text{H}_2\text{SO}_4$  or  $\text{Na}_2\text{CO}_3$ . Aseptically distribute into sterile 50.0mL or 100.0mL bottles with metal screw-caps and rubber seals. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the cultivation of *Ectothiorhodospira marismortui* and *Ectothiorhodospira mobilis*.

#### *Chromatium salexigens* Medium

**Composition** per 4990.0mL:

Solution A .....	4000.0mL
Solution B .....	860.0mL
Solution C (Vitamin $\text{B}_{12}$ solution) .....	5.0mL
Solution D .....	
(Trace elements solution SL-12B) .....	5.0mL
Solution E .....	100.0mL
Solution F .....	20.0mL

pH 7.3  $\pm$  0.2 at 25°C

#### **Solution A:**

**Composition** per 4000.0mL:

$\text{NaCl}$ .....	100.0g
$\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ .....	3.0g
$\text{MgSO}_4$ .....	2.5g
$\text{KH}_2\text{PO}_4$ .....	1.7g
$\text{NH}_4\text{Cl}$ .....	1.7g
$\text{KCl}$ .....	1.7g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	1.25g
Sodium acetate .....	0.5g
$\text{Na}_2\text{S}_2\text{O}_3$ .....	0.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Adjust pH to 6.0. Dispense into a 5-liter flask with four openings at the top (two openings are in a central silicon rubber stopper and two openings are gas-tight screw caps). Add a teflon-coated magnetic stir bar to the flask. Autoclave for 45 min at 15 psi pressure–121°C. Cool to room temperature under 100%  $\text{N}_2$  at 0.05–0.1 atm pressure (use a manometer to measure low pressure).

#### **Solution B:**

**Composition** per 860.0mL:

Distilled/deionized water .....	860.0mL
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**Preparation of Solution B:** Add 860.0mL of distilled/deionized water to a cotton-stoppered flask. Autoclave for 20 min at 15 psi–121°C. Cool to room temperature under 100%  $\text{N}_2$  in an anaerobic jar.

#### **Solution C (Vitamin $\text{B}_{12}$ Solution):**

**Composition** per 5.0mL:

Vitamin $\text{B}_{12}$ .....	1.0mg
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**Preparation of Solution C (Vitamin  $\text{B}_{12}$  Solution):** Add Vitamin  $\text{B}_{12}$  to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Filter sterilize.

**Solution D (Trace Elements Solution SL-12B):****Composition** per liter:

Disodium ethylenediamine-tetraacetate (Disodium EDTA) .....	3.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
ZnCl <sub>2</sub> .....	42.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	18.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg

**Preparation of Solution D (Trace Elements Solution SL-12B):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi–121°C.

**Solution E:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

**Solution F:****Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Dispense into a screw-capped bottle. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Saturate cooled solution A under 100% CO<sub>2</sub> at 0.05–0.1 atm pressure for 30 min with magnetic stirring. Add 860.0mL of solution B, 5.0mL of solution C, 5.0mL of solution D, 100.0mL of solution E, and 20.0mL of solution F through one of the screw-capped openings under 95% N<sub>2</sub> and 5% CO<sub>2</sub> with magnetic stirring. Adjust pH to 7.3 with sterile 2M HCl or sterile 2M Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically and anaerobically distribute the medium through the medium outlet tube into sterile 100.0mL bottles under 95% N<sub>2</sub> and 5% CO<sub>2</sub> at 0.05–0.1 atm pressure. Leave a small gas bubble in each bottle to accommodate pressure changes. After 24 hr, the iron in the medium will precipitate out of solution as black flocs.

**Use:** For the growth and maintenance of *Chromatium sal-exigens*.

***Chromatium tepidum* Medium****Composition** per 1001.0mL:

NH <sub>4</sub> Cl .....	400.0mg
NaCl .....	400.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	200.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
Disodium ethylenediamine-tetraacetate (Disodium EDTA) .....	10.0mg
Ammonium acetate (or sodium pyruvate) .....	0.5mg
KH <sub>2</sub> PO <sub>4</sub> .....	0.5mg
NaHCO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition** per 20.0mL:

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 20.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solutions:****Composition** per liter:

Disodium ethylenediamine-tetraacetate (Disodium EDTA) .....	5.2g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	188.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	25.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg

**Preparation of Trace Elements Solutions:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile NaHCO<sub>3</sub> solution and 20.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 7.0. Distribute into sterile screw-capped tubes or bottles so that medium completely fills container.

**Use:** For the growth and maintenance of *Chromatium tepidum*.

***Chromatium/Thiocapsa* Medium****Composition** per 1060.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Solution A .....	20.0mL
Solution B .....	20.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL
Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of Solution A:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution B:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

$\text{Na}_2\text{S}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$  ..... 10.0g

**Preparation of Solution C:** Add  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution D:

**Composition** per 100.0mL:

Sodium malate ..... 10.0g

**Preparation of Solution D:** Add sodium malate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution:

**Composition** per liter:

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  ..... 2.7g

$\text{H}_3\text{BO}_3$  ..... 0.1g

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g

$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  ..... 50.0mg

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 5.0mg

$\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except solution A, solution B, solution C, and solution D, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 7.0–7.2 with  $\text{H}_3\text{PO}_4$ . Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.2mL of sterile solution A, 0.2mL of sterile solution B, 0.1mL of sterile solution C, and 0.1mL of sterile solution D for each 10.0mL of medium. Mix thoroughly. Use immediately.

**Use:** For the cultivation of *Chlorobium limnicola* and *Chromatium* species.

### *Chromobacterium Medium*

**Composition** per liter:

$\text{NaCl}$  ..... 30.0g

$\text{MgCl}_2$  ..... 10.8g

$\text{MgSO}_4$  ..... 5.4g

Peptone ..... 5.0g

$\text{CaCl}_2$  ..... 1.0g

$\text{KCl}$  ..... 0.7g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Chromobacterium* species and *Alteromonas luteoviolacea*.

### Chu's No. 10 Medium

**Composition** per liter:

Agar ..... 15.0g

$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  ..... 0.232g

$\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$  ..... 0.044g

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.025g

$\text{Na}_2\text{CO}_3$  ..... 0.02g

$\text{K}_2\text{HPO}_4$  ..... 0.01g

Citric acid ..... 3.5mg

Ferric citrate ..... 3.5mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Anabaena* species and *Plectomena boryanum*.

### Chu's No. 10 Medium, Modified

**Composition** per liter:

Agar ..... 15.0g

$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  ..... 0.232g

$\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$  ..... 0.044g

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.025g

$\text{Na}_2\text{CO}_3$  ..... 0.02g

$\text{K}_2\text{HPO}_4$  ..... 0.01g

Citric acid ..... 3.5mg

Ferric citrate ..... 3.5mg

Metal solution ..... 1.0mL

#### Metal Solution:

**Composition** per liter:

$\text{H}_3\text{BO}_3$  ..... 2.4g

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 1.4g

$\text{ZnCl}_2$  ..... 0.4g

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 0.02g

$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 0.1mg

**Preparation of Metal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Anabaena* species and *Plectomena boryanum*.

### Chu's No. 11 Medium, Modified

**Composition** per liter:

$\text{NaNO}_3$  ..... 1.5g

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.08g

$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$  ..... 0.06g

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 0.04g

$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$  ..... 0.04g

$\text{Na}_2\text{CO}_3$  ..... 0.02g

Citric acid ..... 6.0mg

Ferric ammonium citrate ..... 6.0mg

EDTA ..... 1.0mg

Seawater ..... 999.0mL

Trace metal solution A5 with cobalt ..... 1.0mL

pH 7.5 ± 0.2 at 25°C

#### Trace Metal Solution A5 with Cobalt:

**Composition** per liter:

$\text{H}_3\text{BO}_3$  ..... 2.86g

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 1.81g

$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 0.39g

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.222g

$\text{CuSO}_4 \cdot \text{H}_2\text{O}$  ..... 0.079g

$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  ..... 0.049g

**Preparation of Trace Metal Solution A5 with Cobalt:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to seawater and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of cyanobacteria from marine habitats.

**CIN Agar**  
(*Yersinia* Selective Agar)  
(Cefsulodin Irgasan® Novobiocin Agar)

**Composition per liter:**

Mannitol	20.0g
Agar	12.0g
Pancreatic digest of gelatin	10.0g
Beef extract	5.0g
Peptic digest of animal tissue	5.0g
Sodium pyruvate	2.0g
Yeast extract	2.0g
NaCl	1.0g
Sodium deoxycholate	0.5g
Neutral Red	0.03g
Cefsulodin	0.015g
Irgasan (triclosan)	4.0mg
Novobiocin	2.5mg
Crystal Violet	1.0mg

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except cefsulodin and novobiocin, to distilled/deionized water and bring volume to 1.0L. Heat, mixing continuously, until boiling. Do not autoclave. Cool to 45°–50°C. Aseptically add cefsulodin and novobiocin. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and differentiation of *Yersinia enterocolitica* from a variety of nonclinical specimens based on mannitol fermentation. *Yersinia enterocolitica* appears as “bull’s eye” colonies with deep red centers surrounded by a transparent periphery.

**Citrate Medium, Koser’s Modified**

**Composition per liter:**

NaCl	5.0g
Citric acid	2.0g
(NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub>	1.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria based on their ability to utilize citrate as a carbon source.

**Citrate Phosphate Buffered  
Glucose Medium**

**Composition per liter:**

Solution A	750.0mL
Solution C	200.0mL
Solution B	50.0mL

pH 3.5 ± 0.2 at 25°C

**Solution A:**

**Composition per 750.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3.0g
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Citric acid, anhydrous	1.92g
Na <sub>2</sub> HPO <sub>4</sub>	1.23g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
KCl	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 750.0mL. Mix thoroughly.

**Solution B:**

**Composition per 50.0mL:**

Glucose	10.0g
Yeast extract	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**Solution C:**

**Composition per 200.0mL:**

Agarose (electrophoresis grade)	6.0g
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**Preparation of Solution C:** Add agarose to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly.

**Preparation of Medium:** Prepare solutions A, B, and C. Autoclave solutions separately for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Combine solutions A, B, and C. Mix thoroughly. Immediately distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Acidiphilium organovorum*.

**CK Agar**

**Composition per liter:**

Agar	15.0g
Glucose	5.0g
KNO <sub>3</sub>	2.0g
CaCl <sub>2</sub>	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.5g
K <sub>2</sub> HPO <sub>4</sub>	0.25g
Ferric citrate	0.02g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

**CK1 Medium**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
KNO <sub>3</sub>	2.0g
CaCl <sub>2</sub>	1.4g
Ferric citrate	0.02g
Glucose solution	100.0mL
K <sub>2</sub> HPO <sub>4</sub> solution	10.0mL

**Glucose Solution:**

**Composition per 100.0mL:**

D-Glucose	10.0g
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**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**K<sub>2</sub>HPO<sub>4</sub> Solution:****Composition** per 10.0mL:K<sub>2</sub>HPO<sub>4</sub> ..... 2.5mg

**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except glucose solution and K<sub>2</sub>HPO<sub>4</sub> solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add sterile glucose solution and K<sub>2</sub>HPO<sub>4</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of myxobacteria.

***Clostridium beijerinckii* Agar****Composition** per liter:

Agar ..... 20.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 5.0g  
Proteose peptone No. 3 ..... 5.0g  
Sodium thioglycolate ..... 5.0g  
Yeast extract ..... 5.0g  
Polygalacturonic acid solution ..... 50.0mL  
pH 7.5 ± 0.2 at 25°C

**Polygalacturonic Acid Solution:****Composition** per liter:

Polygalacturonic acid (or pectin) ..... 4.6g

**Preparation of Polygalacturonic Acid Solution:**

Dissolve polygalacturonic acid or pectin in small amounts of distilled/deionized water neutralized with 10% NaOH to pH 7.2. Mix intensively. Bring volume to 50.0mL with distilled/deionized water.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Clostridium beijerinckii*.

***Clostridium bryantii* Medium****Composition** per liter:

NaCl ..... 21.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.1g  
Na<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
KCl ..... 0.5g  
NH<sub>4</sub>Cl ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
Resazurin ..... 1.0mg  
NaHCO<sub>3</sub> solution ..... 20.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 20.0mL  
Sodium caproate solution ..... 20.0mL  
Vitamin solution ..... 20.0mL  
Trace elements solution SL-10 ..... 1.0mL  
pH 7.2 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition** per 20.0mL:NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 20.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Sodium Caproate Solution:****Composition** per 20.0mL:

Sodium caproate ..... 1.4g

**Preparation of Sodium Caproate Solution:** Add sodium caproate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition** per 20.0mL:

Thiamine·HCl ..... 100.0μg  
p-Aminobenzoic acid ..... 40.0μg  
D(+)-Biotin ..... 10.0μg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:**

Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, sodium caproate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 919.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 20.0mL of sterile sodium caproate solution, and 1.0mL of sterile trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles.

**Use:** For the cultivation and maintenance of *Syntrophospira bryantii*.

***Clostridium cellobioparum* Agar****Composition** per 1025.0mL:

Ground meat, fat free ..... 500.0g  
Pancreatic digest of casein ..... 30.0g  
Agar ..... 15.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 5.0g  
Yeast extract ..... 5.0g  
Glucose ..... 4.0g  
Cellobiose ..... 1.0g  
Maltose ..... 1.0g

Soluble starch.....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
Resazurin .....	1.0mg
NaOH (1N solution).....	25.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Remove fat and connective tissue from lean beef or horse meat. Grind meat finely. Add ground meat to 25.0mL of 1N NaOH. Add 1.0L of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 15 min while stirring. Cool to room temperature. Skim fat off surface. Filter suspension and retain the filtrate and the meat particles. Bring volume of filtrate to 1.0L with distilled/deionized water. Add remaining components, except L-cysteine-HCl·H<sub>2</sub>O. Mix thoroughly. Gently heat and bring to boiling. Cool to 50°–55°C. Add L-cysteine-HCl·H<sub>2</sub>O. Adjust pH to 7.0. Distribute 7.0mL of agar into tubes containing meat particles (use 1 part meat particles to 4–5 parts fluid). Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Clostridium cellobioparum*.

#### *Clostridium cellobioparum* Medium

**Composition per 1010.0mL:**

NaCl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg
Rumen fluid, clarified .....	300.0mL
Cellobiose solution .....	50.0mL
NaHCO <sub>3</sub> solution .....	30.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
L-Cysteine-HCl solution.....	10.0mL
pH 6.8 ± 0.2 at 25°C	

#### **Cellobiose Solution:**

**Composition per 50.0mL:**

D-Cellobiose.....	5.0g
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**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

#### **NaHCO<sub>3</sub> Solution:**

**Composition per 30.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 20.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **L-Cysteine-HCl Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl.....	0.25g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume

to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except cellobiose solution, NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and L-cysteine-HCl solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile cellobiose solution, 30.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile L-cysteine-HCl solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles.

**Use:** For the cultivation and maintenance of *Clostridium cellobioparum* and *Clostridium polysaccharolyticum*.

#### *Clostridium* Cellulolytic Medium

**Composition per liter:**

Agar .....	20.0g
Cellulose.....	7.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	2.9g
Yeast extract .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
FeSO <sub>4</sub> .....	1.25g
L-Cysteine-HCl·H <sub>2</sub> O .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	2.0mg
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except L-cysteine-HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat to boiling. Adjust pH to 7.5. Prereduce under 100% N<sub>2</sub>. Add L-cysteine-HCl·H<sub>2</sub>O. Distribute into tubes under 100% N<sub>2</sub>. Cap tubes with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Clostridium cellulolyticum* and other bacteria that can degrade cellulose.

#### *Clostridium* Cellulose Medium

**Composition per liter:**

Agar .....	20.0g
Filter paper (or 5.0g Avicel) .....	10.0g
CaCO <sub>3</sub> .....	5.0g
Polypeptone™ .....	5.0g
Na <sub>2</sub> CO <sub>3</sub> ·10H <sub>2</sub> O .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.2g
Yeast extract .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.15g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.0mg
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Clostridium cellulolyticum* and other bacteria that can degrade cellulose.

#### *Clostridium cellulovorans* Medium

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	1.0g
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NH <sub>4</sub> Cl.....	1.0g
KCl.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Pancreatic digest of casein.....	0.5g
Yeast extract.....	0.5g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
Cellulose, MN 300	
or cellobiose solution.....	50.0mL
Na <sub>2</sub> CO <sub>3</sub> solution.....	30.0mL
Rumen fluid, clarified.....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O.....	20.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.0 ± 0.2 at 25°C	

**Cellobiose Solution:****Composition per 50.0mL:**

Cellulose, MN 300 or D-cellobiose.....	5.0g
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**Preparation of Cellobiose Solution:** Add cellulose or cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition per 30.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	1.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 20.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.15g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Add components, except cellobiose solution, Na<sub>2</sub>CO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile cellobiose solution, 30.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, and 20.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles.

**Use:** For the cultivation and maintenance of *Clostridium cellulovorans*.

***Clostridium formicoaceticum* Agar****Composition per liter:**

Agar.....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	10.0g
Yeast extract.....	5.0g
Sodium thioglycolate.....	0.75g
Pyridoxine-HCl.....	1.0mg
Resazurin.....	1.0mg
Fructose solution.....	50.0mL
NaHCO <sub>3</sub> solution.....	30.0mL
Trace elements solution SL-4.....	10.0mL
pH 8.0 ± 0.2 at 25°C	

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

**Trace Elements Solutions SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Trace Elements Solution SL-4:**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Fructose Solution:****Composition per 50.0mL:**

Fructose.....	5.0g
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**Preparation of Fructose Solution:** Add fructose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

**NaHCO<sub>3</sub> Solution:****Composition per 30.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except fructose solution and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to 50°C while sparging with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile fructose solution and 30.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 8.0. Aseptically and anaerobically pour into sterile Petri dishes or distribute into sterile screw-capped bottles under 100% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Clostridium formicoaceticum*.

***Clostridium halophilum* Medium****Composition per liter:**

Solution A.....	900.0mL
Solution B.....	80.0mL

Solution C .....	10.0mL
Solution D .....	10.0mL
pH 8.3 ± 0.2 at 25°C	

**Solution A:****Composition per 900.0mL:**

NaCl .....	60.0g
Betaine .....	5.86g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
L-Alanine .....	2.2g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	25.0mg
Resazurin .....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	15.0μg
Wolfe's vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution B:****Composition per 80.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 20 min.

**Solution C:****Composition per 10.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	0.358g
KH <sub>2</sub> PO <sub>4</sub> .....	0.223g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically combine 900.0mL of cooled solution A with 80.0mL of sparged solution B. Mix thoroughly. Adjust pH to 8.3. Anaerobically distribute 9.8mL volumes into anaerobe tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.1mL of sterile solution C and 0.1mL of sterile solution D to each tube. Mix thoroughly.

**Use:** For the cultivation of *Clostridium halophilum*.

**Clostridium hydroxybenzoicum Medium****Composition per 1055.0mL:**

NaCl .....	10.0g
Yeast extract .....	10.0g
L-Arginine-HCl .....	2.1g
L-Lysine-HCl .....	1.8g
Glycine .....	0.75g
NH <sub>4</sub> Cl .....	0.5g
L-Cysteine-HCl .....	0.4g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
MgCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.025g
Resazurin .....	1.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05mg
Sodium/potassium phosphate buffer (0.02M solution, pH 7.0) .....	1.0L
Wolfe's vitamin solution .....	50.0mL
Wolfe's mineral solution .....	5.0mL
pH 7.0 ± 0.2 at 25°C	

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g



NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Combine components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Clostridium hydroxybenzoicum*.

### *Clostridium kluyveri* Agar

#### Composition per 100.0mL:

Potassium acetate .....	1.0g
Sodium thioglycolate .....	50.0mg
K <sub>2</sub> HPO <sub>4</sub> .....	31.0mg
NH <sub>4</sub> Cl .....	25.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	23.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.2mg
Agar .....	50.0μg
Resazurin .....	50.0μg
<i>p</i> -Aminobenzoic acid .....	20.0μg
Biotin .....	10.0μg
CaCO <sub>3</sub> .....	variable
Ethanol .....	2.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except sodium thioglycolate, ethanol, and CaCO<sub>3</sub>, to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool rapidly to 50°C. Add sodium thioglycolate and ethanol. Distribute into tubes containing a small amount of CaCO<sub>3</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store anaerobically.

**Use:** For the cultivation and maintenance of *Clostridium kluyveri*.

### *Clostridium lentocellum* Agar

#### Composition per 1201.0mL:

Agar .....	30.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.65g
NH <sub>4</sub> SO <sub>4</sub> .....	1.6g
Yeast extract .....	1.0g
NaCl .....	0.96g
L-Cysteine-HCl .....	0.5g
CaCl <sub>2</sub> .....	96.0mg
MgSO <sub>4</sub> .....	96.0mg
Cellulose suspension .....	200.0mL
Resazurin (0.1% solution) .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Cellulose Suspension:

##### Composition per 100.0mL:

Whatman CF cellulose powder .....	4.0g
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**Preparation of Cellulose Suspension:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Clostridium lentocellum* and other *Clostridium* species.

### *Clostridium litoreum* Medium

#### Composition per liter:

Solution A .....	900.0mL
Solution B .....	80.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL

pH 8.3 ± 0.2 at 25°C

#### Solution A:

##### Composition per 900.0mL:

NaCl .....	10.0g
Betaine .....	5.86g
L-Alanine .....	2.2g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	25.0mg
Resazurin .....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	15.0μg
Wolfe's vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

#### Wolfe's Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution B:****Composition** per 80.0mL:

NaHCO<sub>3</sub>..... 5.0g

**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 20 min.

**Solution C:****Composition** per 10.0mL:

K<sub>2</sub>HPO<sub>4</sub>..... 0.358g

KH<sub>2</sub>PO<sub>4</sub>..... 0.223g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O..... 0.3g

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically combine 900.0mL of cooled solution A with 80.0mL of sparged solution B. Mix thoroughly. Adjust pH to 8.3. Anaerobically distribute 9.8mL volumes into anaerobe tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.1mL of sterile solution C and 0.1mL of sterile solution D to each tube. Mix thoroughly.

**Use:** For the cultivation of *Clostridium ljungdahlii*.

***Clostridium ljungdahlii* Medium  
(DSMZ Medium 879)**

**Composition** per liter:

NH<sub>4</sub>Cl..... 1.0g

Yeast extract..... 1.0g

NaCl..... 0.8g

MgSO<sub>4</sub>·7H<sub>2</sub>O..... 0.2g

KCl..... 0.1g

KH<sub>2</sub>PO<sub>4</sub>..... 0.1g

CaCl<sub>2</sub>·2H<sub>2</sub>O..... 0.02g

Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O..... 0.20mg

Fructose solution..... 50.0mL

Trace elements solution..... 10.0mL

Vitamin solution..... 10.0mL

NaHCO<sub>3</sub> solution..... 10.0mL

Cysteine solution..... 10.0mL

Na<sub>2</sub>S·9H<sub>2</sub>O solution..... 10.0mL

pH 5.9 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition** per 10.0mL:

NaHCO<sub>3</sub>..... 1.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Fructose Solution:****Composition** per 50.0mL:

Fructose..... 5.0g

**Preparation of Fructose Solution:** Add fructose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**L-Cysteine Solution:****Composition** per 10.0mL:

L-Cysteine·HCl·H<sub>2</sub>O..... 0.3g

**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O..... 3.0g

Nitrilotriacetic acid..... 1.5g

NaCl..... 1.0g

MnSO<sub>4</sub>·2H<sub>2</sub>O..... 0.5g

CoSO<sub>4</sub>·7H<sub>2</sub>O..... 0.18g

ZnSO<sub>4</sub>·7H<sub>2</sub>O..... 0.18g

CaCl<sub>2</sub>·2H<sub>2</sub>O..... 0.1g

FeSO<sub>4</sub>·7H<sub>2</sub>O..... 0.1g

NiCl<sub>2</sub>·6H<sub>2</sub>O..... 0.025g

KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O..... 0.02g

H<sub>3</sub>BO<sub>3</sub>..... 0.01g

Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O..... 0.01g

CuSO<sub>4</sub>·5H<sub>2</sub>O..... 0.01g

Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition** per liter:

Pyridoxine·HCl..... 10.0mg

Thiamine·HCl·2H<sub>2</sub>O..... 5.0mg

Riboflavin..... 5.0mg

Nicotinic acid..... 5.0mg

D-Ca-pantothenate..... 5.0mg

p-Aminobenzoic acid..... 5.0mg

Lipoic acid..... 5.0mg

Biotin..... 2.0mg

Folic acid..... 2.0mg

Vitamin B<sub>12</sub>..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, fructose solution, cysteine solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 10 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL fructose solution, 10.0mL NaHCO<sub>3</sub> solution, 10.0mL cysteine solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL vitamin solution, and 10.0mL trace elements solu-

tion SL-10. Mix thoroughly. Final pH is 5.9. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Clostridium ljungdahlii*.

### *Clostridium M1 Medium*

**Composition** per liter:

NaCl.....	10.0g
Betaine-H <sub>2</sub> O.....	6.0g
NaHCO <sub>3</sub> .....	5.0g
L-Alanine.....	2.2g
NH <sub>4</sub> Cl.....	1.0g
Yeast extract.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	25.0mg
Resazurin.....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	15.0μg
Phosphate solution.....	100.0mL
Vitamin solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.3 ± 0.2 at 25°C	

### **Phosphate Solution:**

**Composition** per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.358g
KH <sub>2</sub> PO <sub>4</sub> .....	0.223g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

### **Trace Elements Solution SL-10:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thorough-

ly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Add components, except phosphate solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 100.0mL of sterile phosphate solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Clostridium halophilum* and *Clostridium littoralis*.

### *Clostridium papyrosolvens Medium*

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	1.65g
NH <sub>4</sub> Cl.....	1.0g
Yeast extract.....	0.6g
L-Cysteine-HCl.....	0.5g
Resazurin.....	1.0mg
Seawater, filtered.....	200.0mL
Mineral salt solution.....	150.0mL
Cellobiose solution.....	50.0mL
pH 7.2 ± 0.2 at 25°C	

### **Mineral Salt Solution:**

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
NaCl.....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.8g

**Preparation of Mineral Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Cellobiose Solution:**

**Composition** per 50.0mL:

D-Cellobiose.....	5.0g
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**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize.

**Preparation of Medium:** Add components, except cellobiose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 7.2 with 5N NaOH. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile cellobiose solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Clostridium papyrosolvens*.

### *Clostridium pfennigii Medium*

**Composition** per 1001.0mL:

Solution A.....	890.0mL
Solution B.....	100.0mL
Solution C.....	10.0mL
Solution D.....	1.0mL
pH 7.0–7.2 at 25°C	

### **Solution A:**

**Composition** per 890.0mL:

Sodium vanillate.....	2.0g
Yeast extract.....	2.0g
Resazurin.....	1.0mg

Rumen fluid, clarified .....	267.0mL
Mineral solution .....	50.0mL
Vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Adjust pH to 6.9. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min. Distribute 8.9mL into anaerobic tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Mineral Solution:

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	10.0g
NaCl .....	8.0g
NH <sub>4</sub> Cl .....	8.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	6.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

**Composition** per liter:

Pyridoxine·HCl .....	6.2mg
Nicotinic acid .....	2.5mg
<i>p</i> -Aminobenzoic acid .....	1.25mg
Thiamine·HCl .....	1.25mg
Pantothenic acid .....	0.62mg
Biotin .....	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

#### Solution B:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution C:

**Composition** per 10.0mL:

L-Cysteine .....	0.24g
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**Preparation of Solution C:** Add L-cysteine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Solution D:

**Composition** per 1.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	78.0mg
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To each tube containing 8.9mL of sterile solution A, add (using a syringe) 1.0mL of sterile solution B, 0.1mL of sterile solution C, and 0.01mL of sterile solution D.

**Use:** For the cultivation and maintenance of *Clostridium pfennigii*.

#### Clostridium propionicum Medium

**Composition** per 1007.5mL:

Yeast extract .....	4.0g
L-Alanine .....	3.0g
Peptone .....	3.0g
L-Cysteine·HCl .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.018g
Resazurin .....	1.0mg
Potassium phosphate buffer solution, 1M, pH 7.1 .....	5.0mL
CaSO <sub>4</sub> , saturated solution .....	2.5mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 7.1. Sparge with 100% N<sub>2</sub> for 20 min. Distribute into tubes or bottles under 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Clostridium propionicum*.

#### Clostridium sticklandii Medium

**Composition** per liter:

Yeast extract .....	5.0g
L-Arginine·HCl .....	2.0g
L-Lysine·HCl .....	2.0g
NH <sub>4</sub> Cl .....	2.0g
Sodium formate .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.75g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
Na <sub>2</sub> S·H <sub>2</sub> O solution .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.1mL of sterile Na<sub>2</sub>S·H<sub>2</sub>O solution to each 10.0mL of medium.

**Use:** For the cultivation of *Clostridium sticklandii*.

***Clostridium termitidis* Medium****Composition per liter:**

NaCl .....	1.0g
KCl .....	0.5g
Yeast extract .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
Trace elements solution SL-10 .....	1.0mL
Cellobiose solution .....	50.0mL
NaHCO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Cellobiose Solution:****Composition per 50.0mL:**

D-Cellobiose .....	5.0g
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**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize.

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	4.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except cellobiose solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, and bring volume to 920.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> until pH reaches below 6.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile cellobiose solution, 20.0mL of sterile NaHCO<sub>3</sub> solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Clostridium termitidis*.

***Clostridium thermoaceticum* Medium (TYE-CO)****(DSMZ Medium 316)****Composition per liter:**

Trypticase .....	10.0g
Yeast extract .....	3.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	2.8g
NH <sub>4</sub> Cl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	5.0mL

pH 7.0 ± 0.2 at 25°C

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 100%

CO<sub>2</sub>. Aseptically and anaerobically add 10.0mL vitamin solution, and 10.0mL of sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Moorella thermoacetica*=*Clostridium thermaceticum*.

### ***Clostridium thermocellum* Medium (LMG Medium 42)**

**Composition** per liter:

Agar .....	30.0g
Cellulose .....	10.0g
Sodium-beta-glycerophosphate .....	6.0g
K <sub>2</sub> HPO <sub>4</sub> .....	5.5g
Yeast extract .....	4.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.6g
KH <sub>2</sub> PO <sub>4</sub> .....	1.43g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.13g
Glutathione .....	0.25g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1mg
Resazurin .....	1.0mg

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L under 95% N<sub>2</sub> + 5% CO<sub>2</sub> gas atmosphere. Mix thoroughly and sparge with 95% N<sub>2</sub> + 5% CO<sub>2</sub> gas. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Clostridium thermocellum*.

### ***Clostridium thermohydrosulfuricum* Medium**

**Composition** per liter:

Sucrose .....	10.0g
Pancreatic digest of casein .....	10.0g
Yeast extract .....	2.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> SO <sub>3</sub> .....	0.2g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.08g
Resazurin .....	1.0mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 15 min. Autoclave for 30 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Clostridium thermohydrosulfuricum*, *Clostridium thermosaccharolyticum*, *Thermoanaerobacter ethanolicus*, and *Thermoanaerobacter thermohydrosulfuricus*.

### ***Clostridium thermosuccinogenes* Medium**

**Composition** per 1011.0mL:

Inulin .....	5.0g
NaCl .....	1.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
KCl .....	0.3g
NH <sub>4</sub> Cl .....	0.27g
KH <sub>2</sub> PO <sub>4</sub> .....	0.21g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Na <sub>2</sub> SO <sub>4</sub> .....	0.1g
Resazurin .....	1.0mg
Na <sub>2</sub> HPO <sub>4</sub> solution .....	20.0mL

Vitamin solution .....	10.0mL
Yeast extract solution .....	10.0mL
Casamino acids solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

### **Na<sub>2</sub>HPO<sub>4</sub> Solution:**

**Composition** per 20.0mL:

Na <sub>2</sub> HPO <sub>4</sub> .....	2.66g
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**Preparation of Na<sub>2</sub>HPO<sub>4</sub> Solution:** Add Na<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Yeast Extract Solution:**

**Composition** per 10.0mL:

Yeast extract .....	0.03 g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

### **Casamino Acids Solution:**

**Composition** per 10.0mL:

Casamino acids .....	0.03 g
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**Preparation of Casamino Acids Solution:** Add casamino acids to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

### **NaHCO<sub>3</sub> Solution:**

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	1.0mg
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.15mg
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Trace Elements Solution SL-10:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>HPO<sub>4</sub> solution, vitamin solution, yeast extract solution, casamino acids solution, NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile Na<sub>2</sub>HPO<sub>4</sub> solution, 10.0mL of sterile vitamin solution, 10.0mL of sterile yeast extract solution, 10.0mL of sterile casamino acids solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL of sterile trace elements solution SL-10. Aseptically and anaerobically distribute into tubes or bottles.

**Use:** For the cultivation and maintenance of *Clostridium thermosuccinogenes*.

#### *Clostridium thermosulfurogenes* Medium

**Composition** per 1015.0mL:

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	5.3g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5mg
Resazurin .....	1.0mg
Glucose solution .....	50.0mL
Trace elements solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	5.0mL

pH 6.0 ± 0.2 at 25°C

#### **Glucose Solution:**

**Composition** per 50.0mL:

D-Glucose .....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Elements Solution:**

**Composition** per liter:

Nitilotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.02g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water.

Adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 7.0 with 1N HCl before use.

**Preparation of Medium:** Add components, except glucose solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, and bring volume to 940.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile glucose solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into tubes or bottles.

**Use:** For the cultivation and maintenance of *Thermoanaerobacterium thermosulfurogenes*.

#### **CM4 Medium**

**Composition** per liter:

Cellobiose .....	6.0g
Yeast extract .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
NaCl .....	1.0g
MgCl <sub>2</sub> .....	0.75g
Sodium thioglycolate .....	0.5g
CaCl <sub>2</sub> .....	0.0132g
Resazurin (1.0% solution) .....	0.2mL
FeSO <sub>4</sub> (1.25% solution) .....	0.1mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until boiling. Boil until color changes from red to colorless, indicating a reduced state. Cool. Distribute into tubes or flasks under 97% N<sub>2</sub> + 3% H<sub>2</sub>. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Clostridium* species and other bacteria that can utilize cellobiose as a carbon source.

**Colby and Zatman Agar****Composition per liter:**

Agar, noble.....	20.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.62g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
NaCl.....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.05g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	70.0µg
H <sub>3</sub> BO <sub>3</sub> .....	10.0µg
MnSO <sub>4</sub> ·5H <sub>2</sub> O.....	10.0µg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	10.0µg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.0µg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0µg
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	1.0mg
Trimethylamine solution.....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trimethylamine Solution****Composition per 10.0mL:**

Trimethylamine.....	1.0g
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**Preparation of Trimethylamine Solution:** Add trimethylamine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except trimethylamine solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL of sterile trimethylamine solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Aminobacter aminovorans*, *Bacillus* species, *Hyphomicrobium aestuarii*, *Hyphomicrobium facilis*, *Hyphomicrobium* species, *Hyphomicrobium variabile*, *Hyphomicrobium zavarzinii*, *Methylobacterium extorquens*, *Methylobacterium* species, and *Methylophilus methylotrophus*.

**Colloidal Chitin Agar****Composition per liter:**

Agar.....	20.0g
Chitin, colloidal.....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
MgSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01g
MnCl <sub>2</sub> .....	1.0mg
ZnSO <sub>4</sub> .....	1.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Micromonospora* species from water, soil, or sediment. For the germination of spores of *Micromonospora* species.

**Cobwellia psychroerythrus Medium****Composition per liter:**

NaCl.....	29.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	8.0g
Pancreatic digest of casein.....	8.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.4g

CaCl <sub>2</sub> ·6H <sub>2</sub> O.....	33.0mg
FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	2.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Aminobacter aminovorans*, *Bacillus* species, *Hyphomicrobium aestuarii*, *Hyphomicrobium facilis*, *Hyphomicrobium* species, *Hyphomicrobium variabile*, *Hyphomicrobium zavarzinii*, *Methylobacterium extorquens*, *Methylobacterium* species, and *Methylophilus methylotrophus*.

**Cook's Cytophaga Agar****Composition per liter:**

Agar.....	10.0g
Pancreatic digest of casein.....	2.0g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Lysobacter antibioticus*, *Lysobacter brunescens*, *Lysobacter enzymogenes*, *Lysobacter gummosus*, and other *Lysobacter* species.

**Coprinus Medium****Composition per 1026mL:**

Agar.....	20.0g
Glucose.....	20.0g
Asparagine.....	2.0g
Pancreatic digest of casein.....	0.75g
Yeast extract.....	0.75g
Malt extract.....	0.60g
Salt solution.....	25.0mL
Thiamine solution.....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Salt Solution:****Composition per 500mL:**

Na <sub>2</sub> HPO <sub>4</sub> .....	45.0g
KH <sub>2</sub> PO <sub>4</sub> .....	20.0g
Ammonium tartrate.....	10.0g
Na <sub>2</sub> SO <sub>4</sub> ·10H <sub>2</sub> O.....	5.6g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 500mL. Mix thoroughly. Filter sterilize.

**Thiamine Solution:****Composition per 100mL:**

Thiamine.....	10.0mg
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**Preparation of Thiamine Solution:** Add thiamine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except salt solution and thiamine solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 25.0mL of sterile salt solution and 1.0mL of sterile thiamine solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.



**Use:** For the cultivation and maintenance of *Coprinus cinereus*, *Dendrophoma obscurans*, and *Trichophyton violaceum*.

### *Coprothermobacter proteolyticus* Medium

#### Composition per 1168.1mL:

Yeast extract	2.0g
Trypticase	2.0g
NaOH solution	1.0L
Gelatin solution	113.0mL
Na <sub>2</sub> S solution	22.6mL
Solution A	10.0mL
Mineral salts solution	10.0mL
Solution B	2.0mL
Resazurin solution	0.5mL

#### NaOH Solution:

##### Composition per liter:

NaOH	4.0g
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**Preparation of NaOH Solution:** Add NaOH to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Gelatin Solution:

##### Composition per 100.0mL:

Gelatin	3.0g
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**Preparation of Gelatin Solution:** Gently heat 100.0mL of distilled/deionized water to 80°C. Sparge with 100% N<sub>2</sub> for 15 min. Add the gelatin. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 10 min. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S Solution:

##### Composition per 100.0mL:

Na <sub>2</sub> S	2.5g
Distilled water	100 ml

**Preparation of Na<sub>2</sub>S Solution:** Gently heat 100.0mL of distilled/deionized water to 100°C. Boil for 5 min. Sparge with 100% N<sub>2</sub> for 15 min. Add the Na<sub>2</sub>S. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 10 min. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution A:

##### Composition per liter:

NH <sub>4</sub> Cl	100.0g
MgCl <sub>2</sub> ·H <sub>2</sub> O	100.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	40.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4 with HCl.

#### Mineral Salts Solution:

##### Composition per liter:

EDTA·2H <sub>2</sub> O	0.5g
CoCl <sub>2</sub> ·H <sub>2</sub> O	0.15g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
ZnCl <sub>2</sub>	0.1g
AlCl <sub>3</sub> ·H <sub>2</sub> O	40mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	30mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	20mg
NiSO <sub>4</sub> ·H <sub>2</sub> O	20mg
H <sub>2</sub> SeO <sub>3</sub>	10mg
H <sub>3</sub> BO <sub>4</sub>	10mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O	10mg

**Preparation of Mineral Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3 with HCl.

#### Solution B:

##### Composition per liter:

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	200.0g
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**Preparation of Solution B:** Add K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Resazurin Solution:

##### Composition per 100.0mL:

Resazurin	0.2g
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Sparge 1.0L of NaOH solution with 100% CO<sub>2</sub> for 30 min. Add 2.0g of yeast extract and 2.0g of Trypticase. Mix thoroughly. Add 10.0mL of solution A, 2.0mL of solution B, 0.5mL of resazurin solution, and 10.0mL of mineral salts solution with pipets which have been flushed for a few times with 100% N<sub>2</sub>. Mix thoroughly. Anaerobically distribute 9.0mL volumes into anaerobic tubes fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. One hour prior to inoculation, add 1.0mL of sterile gelatin solution and 0.2mL of sterile Na<sub>2</sub>S solution to each 9.0mL of medium.

**Use:** For the cultivation of *Coprothermobacter proteolyticus*.

### Cornmeal Agar (CMA)

#### Composition per liter:

Agar	20.0g
Cornmeal polenta	15.0g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add cornmeal polenta to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 30 min. Filter through Whatman #1 filter paper. Add agar to filtrate. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 10 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of many filamentous fungi.

### Cornmeal Agar with Soil Extract

#### Composition per liter:

Cornmeal	50.0g
Agar	7.5g
Soil extract	50.0mL

#### Soil Extract:

##### Composition per 200.0mL:

African Violet soil	77.0g
Na <sub>2</sub> CO <sub>3</sub>	0.2g

**Preparation of Soil Extract:** Add components to 200.0mL of distilled/deionized water. Mix thoroughly. Autoclave for 60 min at 15 psi pressure–121°C. Filter through paper and reserve filtrate.

**Preparation of Medium:** Add cornmeal to distilled/deionized water and bring volume to 800.0mL. Leave overnight in refrigerator. Heat to 60°C for 1 hr. Add 50.0mL of soil extract. Bring volume to 1.0L with distilled/deionized water. Add agar. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Helicodendron tubulosum*, *Microsporium distortum*, *Mortierella humilis*, *Mortierella hygrophila*, *Mortierella minutissima*, and *Nigrospora sphaerica*.

### **Cornmeal *Phytophthora* Isolation Medium No. 1**

**Composition** per liter:

Agar .....	15.0g
Cornmeal, solids from infusion .....	2.0g
Vancomycin .....	0.2g
Pentachloronitrobenzene (PCNB).....	0.1g
Pimaricin .....	0.01g

pH 5.6–6.0 at 25°C

**Preparation of Medium:** Add components, except pimaricin and vancomycin, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add pimaricin and vancomycin. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the cultivation of *Phytophthora* species.

### **Cornmeal *Phytophthora* Isolation Medium No. 2**

**Composition** per liter:

Agar .....	15.0g
Cornmeal, solids from infusion .....	2.0g
Vancomycin .....	0.3g
Pentachloronitrobenzene (PCNB).....	0.025g
Pimaricin .....	5.0mg

pH 5.6–6.0 at 25°C

**Preparation of Medium:** Add components, except pimaricin and vancomycin, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add pimaricin and vancomycin. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the cultivation of *Phytophthora* species.

### **Cornmeal Yeast Extract Seawater Agar (ATCC Medium 2422)**

**Composition** per liter:

Instant Ocean .....	17.5g
Agar .....	15.0g
Yeast extract .....	1.0g
Cornmeal infusion.....	400.0mL

pH 7.2–7.5 at 25°C

**Cornmeal Infusion:**

**Composition** per liter:

Yellow cornmeal .....	50.0g
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**Preparation of Cornmeal Infusion:** Add cornmeal to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling. Simmer for 10 minutes. Filter through cheesecloth. Return volume to 1.0 liter.

**Preparation of Medium:** Add instant ocean, agar, and yeast extract to 400.0mL cornmeal infusion and bring volume to 1.0L with distilled/deionized water. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of fungi.

### ***Corynebacterium* Agar**

**Composition** per liter:

Agar .....	15.0g
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Beef extract .....	10.0g
Peptone .....	10.0g
NaCl .....	5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Brevibacterium helvolum*, *Brevibacterium linens*, *Brochothrix thermosphacta*, *Cellulomonas cellasea*, *Corynebacterium ammoniagenes*, *Corynebacterium callunae*, *Corynebacterium glutamicum*, other *Corynebacterium* species, *Curtobacterium flaccumfaciens*, *Deinococcus radiodurans*, *Microbacterium laevaniformans*, *Mycobacterium vaccae*, *Rhodococcus equi*, *Rhodococcus fascians*, *Sporolactobacillus inulinus*, and *Streptococcus mutans*.

### ***Corynebacterium* Medium with Salt (DSMZ Medium 229)**

**Composition** per liter:

NaCl .....	65.0g
Agar .....	15.0g
Casein peptone, tryptic digest .....	10.0g
Yeast extract .....	5.0g
Glucose .....	5.0g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Nesterenkonia halobia*=*Micrococcus halobius*.

### **Cow Manure Agar**

**Composition** per liter:

Cow manure .....	50.0g
Agar .....	15.0g

**Preparation of Medium:** Add cow manure to tap water and bring volume to 1.0L. Gently heat and bring to boiling. Boil for 1 hr. Filter through cheese cloth. Filter through Whatman filter paper. Bring volume to 1.0L with tap water. Add agar. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Streptomyces* species.

### **CP Medium for *Coprothermobacter proteolyticus***

**Composition** per 1010.0mL:

NaHCO <sub>3</sub> .....	8.4g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.4g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.4g
Resazurin .....	0.5mg
Gelatin solution .....	100.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Wolfe's mineral solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Gelatin Solution:****Composition** per 100.0mL:

Gelatin..... 3.0g

**Preparation of Gelatin Solution:** Add gelatin to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Wolfe's Mineral Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
 Nitrilotriacetic acid ..... 1.5g  
 NaCl ..... 1.0g  
 MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
 KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
 CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g  
 Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Prepare medium anaerobically under 100% CO<sub>2</sub>. Add components, except gelatin solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and Wolfe's mineral solution, to distilled/deionized water and bring volume to 880.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% CO<sub>2</sub>. Sparge with 100% CO<sub>2</sub> for 20 min. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 100.0mL of sterile gelatin solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile Wolfe's mineral solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Coprothermobacter proteolyticus*.

**CT Agar****Composition** per liter:

Agar ..... 20.0g  
 Pancreatic digest of casein ..... 20.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.0g  
 Potassium phosphate  
 buffer (0.02M solution, pH 7.6) ..... 500.0mL  
 pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add agar, pancreatic digest of casein, and MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave agar–pancreatic digest of casein–MgSO<sub>4</sub>·7H<sub>2</sub>O solution and potassium phosphate buffer solution separately for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically combine the two solu-

tions. Aseptically add sterile components. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of myxobacteria.

**CT Medium****Composition** per liter:

Agar ..... 15.0g  
 Pancreatic digest of casein ..... 10.0g  
 Yeast extract ..... 3.5g  
 MgSO<sub>4</sub> ..... 0.96g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Stigmatella aurantiaca*.

**CTT Medium****Composition** per liter:

Agar ..... 15.0g  
 Pancreatic digest of casein ..... 10.0g  
 Tris(hydroxymethyl)aminomethane buffer ..... 1.21g  
 Potassium phosphate  
 buffer (1 mM, pH 7.6) ..... 1.0L  
 Magnesium sulfate solution ..... 10.0mL  
 pH 7.6 ± 0.2 at 25°C

**Magnesium Sulfate Solution:****Composition** per 10.0mL:MgSO<sub>4</sub>·7H<sub>2</sub>O..... 2.0g

**Preparation of Magnesium Sulfate Solution:** Add MgSO<sub>4</sub>·7H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Adjust pH to 7.6. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

**CY Agar****Composition** per liter:

Agar ..... 15.0g  
 Pancreatic digest of casein ..... 3.0g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.0g  
 Yeast extract ..... 1.0g  
 Cyanocobalamin ..... 0.5mg  
 pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

**CYC Medium****Composition** per liter:

Sucrose ..... 30.0g  
 Casamino acids, vitamin free ..... 6.0g  
 NaNO<sub>3</sub> ..... 3.0g  
 Yeast extract ..... 2.0g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 KCl ..... 0.5g

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Antibiotic solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Antibiotic Solution:****Composition per 10.0mL:**

Cycloheximide .....	0.05g
Novobiocin .....	0.025g

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except antibiotic solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile antibiotic solution. Mix thoroughly. Aseptically distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Thermoactinomyces* species.

**CYC Medium,  
Cross and Attwell Modification  
(DSMZ Medium 550)**

**Composition per liter:**

Sucrose .....	30.0g
Agar .....	15.0g
Casamino acids .....	6.1g
NaNO <sub>3</sub> .....	3.0g
Yeast extract .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.5g
Tryptophan .....	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermomonospora curvata*, *Thermobifida fusca*, *Thermoactinomyces vulgaris*, *Saccharopolyspora thermophila*, and *Thermobifida alba*.

**Cyclohexanecarboxylic Acid Agar****Composition per liter:**

Agar, noble .....	15.0g
Cyclohexanecarboxylic acid .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Yeast extract .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	2.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Corynebacterium cyclohexanicum* and *Saccharomyces cerevisiae*.

**Cyclohexanecarboxylic Acid Medium****Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	3.5g
Cyclohexanecarboxylic acid .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g
NaMoO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Alcaligenes faecalis* and other bacteria that can utilize cyclohexanecarboxylic acid as a carbon source.

**Cyclohexanone Medium****Composition per liter:**

NH <sub>4</sub> NO <sub>3</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.0mg
Cyclohexanone .....	1.0mL

**Preparation of Medium:** Add components, except cyclohexanone, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C. Filter sterilize cyclohexanone. Aseptically add 1.0mL of cyclohexanone. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Nocardia* species and other bacteria that can utilize cyclohexanone as a carbon source.

**CYE-ACES Agar  
(DSMZ Medium 585)**

**Composition per liter:**

Solution A .....	490.0mL
Solution B .....	490.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL

pH 6.9 ± 0.1 at 25°C

**Solution A:****Composition per 490mL:**

Yeast extract .....	10.0g
ACES (N-2-acetamido-2-aminoethane-sulfonic acid) .....	10.0g
Activated charcoal .....	2.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Solution B:****Composition per 490mL:**

Agar .....	15.0g
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**Preparation of Solution B:** Add agar to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

#### **Solution C:**

**Composition** per 10mL:

L-Cysteine-HCl·H<sub>2</sub>O ..... 0.4g

**Preparation of Solution C:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Solution D:**

**Composition** per 10mL:

Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> ..... 0.25g

**Preparation of Solution D:** Add Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Heat to 50–55°C to dissolve Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>. Mix thoroughly. Filter sterilize. Store in the dark. Do not use if chemical loses its green color and becomes brown or yellow.

**Preparation of Medium:** Add 10.0mL solution C and then 10.0mL solution D to 490.0mL solution A. Adjust the pH 6.9 ± 0.05 at 50°C by adding 4.0 to 4.5 ml of sterile 1.0 N KOH. The pH of the medium is critical. Finally, add 490.0mL solution B. Mix thoroughly. Swirl medium in flask during dispensing to petri dishes or tubes to keep charcoal suspended. Pour into Petri dishes or aseptically distribute into sterile tubes.

**Use:** For the cultivation of *Afipia clevelandensis*, *Afipia broomeae*, *Afipia felis*, *Legionella pneumophila*, *Legionella longbeachae*, and *Xylella fastidiosa*.

### **CYE Agar (Charcoal Yeast Extract Agar)**

**Composition** per liter:

Agar ..... 17.0g

Yeast extract ..... 10.0g

Charcoal, activated, acid-washed ..... 2.0g

L-Cysteine-HCl·H<sub>2</sub>O solution ..... 10.0mL

Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution ..... 10.0mL

pH 6.9 ± .05 at 50°C

#### **L-Cysteine-HCl·H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

L-Cysteine-HCl·H<sub>2</sub>O ..... 0.4g

**Preparation of L-Cysteine-HCl·H<sub>2</sub>O solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:**

**Composition** per liter:

Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> ..... 0.25g

**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:** Add soluble Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. The soluble Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> must be kept dry and in the dark. Do not use if brown or yellow. Prepare solutions freshly. Do not heat over 60°C to dissolve. The mixture dissolves readily in a 50°C water bath.

**Preparation of Medium:** Add components, except L-cysteine-HCl·H<sub>2</sub>O solution and Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Add 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution and 10.0mL of sterile Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution. Adjust pH to 6.9 at 50°C by adding 4.0–4.5mL of 1.0N KOH. This is a critical step. Mix thor-

oughly. Pour in 20.0mL volumes into sterile Petri dishes. Swirl medium while pouring to keep charcoal in suspension.

**Use:** For the cultivation and maintenance of *Legionella* species and *Tatlockia micdadei*.

### **CYE Agar, Buffered (Charcoal Yeast Extract Agar, Buffered)**

**Composition** per liter:

Agar ..... 17.0g

ACES buffer (N-2-acetamido-2-aminoethane sulfonic acid) ..... 10.0g

Yeast extract ..... 10.0g

Charcoal, activated, acid-washed ..... 2.0g

L-Cysteine-HCl·H<sub>2</sub>O solution ..... 10.0mL

Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution ..... 10.0mL

pH 6.9 ± .05 at 50°C

#### **L-Cysteine-HCl·H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

L-Cysteine-HCl·H<sub>2</sub>O ..... 0.4g

**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:**

**Composition** per liter:

Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> ..... 0.25g

**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:** Add soluble Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. The soluble Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> must be kept dry and in the dark. Do not use if brown or yellow. Prepare solutions freshly. Do not heat over 60°C to dissolve. The mixture dissolves readily in a 50°C water bath.

**Preparation of Medium:** Add components, except L-cysteine-HCl·H<sub>2</sub>O solution and Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Add 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution and 10.0mL of sterile Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution. Adjust pH to 6.9 at 50°C by adding 4.0–4.5mL of 1.0 N KOH. This is a critical step. Mix thoroughly. Pour in 20.0mL volumes into sterile Petri dishes. Swirl medium while pouring to keep charcoal in suspension.

**Use:** For the cultivation and maintenance of *Legionella* species and *Xylella fastidiosa*.

### **CYG Agar**

**Composition** per liter:

Agar ..... 15.0g

Pancreatic digest of casein ..... 3.0g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.0g

Yeast extract ..... 1.0g

Cyanocobalamin ..... 0.5mg

Glucose solution ..... 100.0mL

pH 7.2 ± 0.2 at 25°C

#### **Glucose Solution:**

**Composition** per 100.0mL:

D-Glucose ..... 5.0g

**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### *Cytophaga* Agarase Agar (ATCC Medium 793)

**Composition** per liter:

Agar .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.5g
CaCl <sub>2</sub> ·H <sub>2</sub> O .....	0.02g
Vishniac and Santer trace element mixture .....	0.2mL
pH 7.2 ± 0.2 at 25°C	

**Vishniac and Santer Trace Element Mixture:**

**Composition** per liter:

Ethylenediamine tetraacetic acid (EDTA) .....	50.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	22.0g
CaCl <sub>2</sub> .....	5.54g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.99g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.61g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	1.1g

**Preparation of Vishniac and Santer Trace Element Mixture:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 6.0 with KOH. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.2. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Cytophaga* *flavescens*.

### *Cytophaga* agarovorans Agar (LMG Medium 99)

**Composition** per 1001.0mL:

NaCl .....	30.0g
Agar .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	1.25mg
Glucose solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	1.0mL

**Glucose Solution:**

**Composition** per 10.0mL:

D-Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile glucose solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, and 1.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Cytophaga* *agarovorans*.

### *Cytophaga* fermentans Medium

**Composition** per liter:

NaCl .....	30.0g
Agar .....	5.0g
NaHCO <sub>3</sub> .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.04g
Ferric citrate (4mM solution) .....	5.0mL
Trace elements solution .....	2.0mL
pH 7.0 ± 0.2 at 25°C	

**Trace Elements Solution:**

**Composition** per 100.0mL:

H <sub>3</sub> BO <sub>3</sub> .....	0.28g
MnSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.21g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.075g
Zn(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
Cu(NO <sub>3</sub> ) <sub>2</sub> ·3H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of agar-digesting *Cytophaga* *fermentans*.

### *Cytophaga* hutchinsonii Agar

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.36g
Yeast extract .....	1.0g
Cellobiose solution .....	50.0mL
pH 7.2 ± 0.2 at 25°C	

**Cellobiose Solution:****Composition per 50.0mL:**

D-Cellobiose ..... 6.0g

**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cellobiose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile cellobiose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Cytophaga aurantiaca* and *Cytophaga hutchinsonii*.

***Cytophaga* Marine Medium  
(DSMZ Medium 172)**

**Composition per liter:**

NaCl ..... 24.7g  
Agar ..... 15.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 6.3g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 4.6g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.2g  
Yeast extract ..... 1.0g  
Tryptone ..... 1.0g  
KCl ..... 0.7g  
Sodium bicarbonate solution ..... 10.0mL  
Calcium chloride solution ..... 10.0mL

pH 7.0 ± 0.2 at 25°C

**Sodium Bicarbonate Solution:****Composition per 10.0mL:**NaHCO<sub>3</sub> ..... 0.2g

**Preparation of Sodium Bicarbonate Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Calcium Chloride Solution:****Composition per 10.0mL:**CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.2g

**Preparation of Calcium Chloride Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except sodium bicarbonate and calcium chloride solution to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45–50°C. Aseptically add 10.0mL of sterile bicarbonate solution and 10.0mL sterile calcium chloride solution. Adjust pH to 7.2. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Cellulophaga lytica*, *Cytophaga latercula*, *Marinilabilia salmonicolor*, *Saprospira grandis*, *Cytophaga marinoflava*, *Persicobacter diffluens*, *Flammeovirga aprica*, *Flexibacter tractuosus*, *Microscilla* sp., *Marinilabilia salmonicolor*, *Flexibacter litoralis*, *Flexithrix dorotheae*, and *Cellulophaga lytica*.

***Cytophaga* Medium**

**Composition per liter:**

NaCl ..... 30.0g  
Agar ..... 15.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
NH<sub>4</sub>Cl ..... 1.0g  
Yeast extract ..... 1.0g  
MgSO<sub>4</sub> ..... 0.5g  
CaCl<sub>2</sub> ..... 0.04g  
FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 1.25mg  
NaHCO<sub>3</sub> solution ..... 100.0mL  
Glucose solution ..... 10.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 1.0mL

**Glucose Solution:****Composition per 100.0mL:**

Glucose ..... 10.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 100.0mL:**Na<sub>2</sub>S·9H<sub>2</sub>O ..... 10.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, glucose solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 889.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add sterile NaHCO<sub>3</sub> solution, sterile glucose solution, and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Cytophaga agarovorans*.

**Czapek Agar  
(ATCC Medium 312)**

**Composition per liter:**

Sucrose ..... 30.0g  
Agar ..... 15.0g  
NaNO<sub>3</sub> ..... 3.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g  
KCl ..... 0.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.01g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except sucrose, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Distribute into tubes or flasks. In a separate flask, add sucrose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave both solutions separately for 15 min at 15 psi pressure–121°C. Cool to 50°C. Combine the sterile solutions. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Streptomyces* species. For the cultivation of Actinoplanaceae.

**Czapek Dox Agar, Modified**

**Composition per liter:**

Sucrose ..... 30.0g

Agar .....	12.0g
NaNO <sub>3</sub> .....	2.0g
Magnesium glycerophosphate .....	0.5g
KCl .....	0.5g
K <sub>2</sub> SO <sub>4</sub> .....	0.35g
FeSO <sub>4</sub> .....	0.01g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of numerous fungal species.

### Czapek Dox Broth

**Composition per liter:**

Sucrose .....	30.0g
NaNO <sub>3</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of a variety of fungal and bacterial species that can use nitrate as sole nitrogen source.

### Czapek Solution Agar with Sucrose

**Composition per liter:**

Sucrose .....	200.0g
Agar .....	20.0g
NaNO <sub>3</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of osmophilic fungi.

### Decarboxylase Base, Møller

**Composition per liter:**

Amino acid .....	10.0g
Beef extract .....	5.0g
Peptone .....	5.0g
Glucose .....	0.5g
Bromcresol Purple .....	0.01g
Cresol Red .....	5.0mg
Pyridoxal .....	5.0mg
Mineral oil .....	200.0mL

pH 6.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except mineral oil, to distilled/deionized water and bring volume to 1.0L. For amino acid, use L-arginine, L-lysine, or L-ornithine. Mix thoroughly. Distribute into screw-capped tubes in 5.0mL volumes. Autoclave medium and mineral oil separately for 15 min at 15 psi pressure–121°C. After inoculation, overlay medium with 1.0mL of sterile mineral oil per tube.

**Use:** For the cultivation and differentiation of bacteria based on their ability to decarboxylate the amino acid. Bacteria that decarboxylate arginine, lysine, or ornithine turn the medium turbid purple.

### Decarboxylase Medium Base, Falkow

**Composition per liter:**

Amino acid .....	5.0g
Peptone .....	5.0g
Yeast extract .....	3.0g
Glucose .....	1.0g
Bromcresol Purple .....	0.02g
Mineral oil .....	200.0mL

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except mineral oil, to distilled/deionized water and bring volume to 1.0L. For amino acid, use L-arginine, L-lysine, or L-ornithine. Mix thoroughly. Distribute into screw-capped tubes in 5.0mL volumes. Autoclave medium and mineral oil separately for 15 min at 15 psi pressure–121°C. After inoculation, overlay medium with 1.0mL of sterile mineral oil per tube.

**Use:** For the cultivation and differentiation of bacteria based on their ability to decarboxylate the amino acid. Bacteria that decarboxylate arginine, lysine, or ornithine turn the medium turbid purple.

### Decarboxylase Medium, Ornithine Modified

**Composition per liter:**

L-Ornithine .....	10.0g
Meat peptone .....	5.0g
Yeast extract .....	3.0g
Bromcresol Purple solution .....	5.0mL

pH 5.5 ± 0.2 at 25°C

### Bromcresol Purple Solution:

**Composition per 100.0mL:**

Bromcresol Purple .....	0.2g
Ethanol .....	50.0mL

**Preparation of Bromcresol Purple Solution:** Add Bromcresol Purple to ethanol. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 5.5 with HCl or NaOH. Distribute into screw-capped tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria based on their ability to decarboxylate ornithine. Bacteria that decarboxylate ornithine turn the medium turbid purple.



**Deferribacter Medium  
(DSMZ Medium 935)****Composition per liter:**

NaCl .....	25.0g
Sulfur, powdered .....	10.0g
Na-acetate .....	2.0g
KNO <sub>3</sub> .....	0.5g
NH <sub>4</sub> Cl .....	0.33g
KCl .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
Yeast extract .....	0.15g
NaHCO <sub>3</sub> solution .....	10.0mL
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	0.3g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, NaHCO<sub>3</sub> solution, and sulfur to 980.0mL distilled/deionized water. Gently heat and bring to boiling. Boil for 3 min. Cool to room temperature while sparging

with 100% N<sub>2</sub>. Adjust pH to 7.0. Anaerobically under 100% N<sub>2</sub> distribute into tubes of bottles containing the sulfur (0.1g sulfur per 10mL medium). Autoclave for 20 min at 110°C. Aseptically and anaerobically add 10.0mL sterile vitamin solution and 10.0mL sterile NaHCO<sub>3</sub> solution. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Deferribacter desulfuricans* and *Deferribacter thermophilus*.

**Defined Glucose Medium EMSY-1****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	1.79g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
Citric acid .....	0.5g
NH <sub>4</sub> Cl .....	0.43g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.41g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.04g
NaCl .....	0.03g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	4.84mg
Glucose solution .....	100.0mL
Yeast extract solution .....	10.0mL
TK6-3 solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Glucose Solution:****Composition per 100.0mL:**

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Yeast Extract Solution:****Composition per 10.0mL:**

Yeast extract .....	0.4g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**TK6-3 Solution:****Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.45g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.76g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.31g
H <sub>3</sub> BO <sub>3</sub> .....	0.19g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.17g
KI .....	0.04g
H <sub>2</sub> SO <sub>4</sub> (1N solution) .....	1.0mL

**Preparation of TK6-3 Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution and yeast extract solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool rapidly to 25°C. Aseptically add 100.0mL of sterile glucose solution and 10.0mL of sterile yeast extract solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Xanthomonas campestris*.

**Defined Medium with Povidone Iodine****Composition per 1025.0mL:**

Basal solution .....	1.0L
Solution B .....	10.0mL
Solution C .....	10.0mL
Solution A .....	5.0mL

**Basal Solution:****Composition per liter:**

Agar .....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	4.8g
KH <sub>2</sub> PO <sub>4</sub> .....	4.4g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

**Preparation of Basal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution A:****Composition per 100.0mL:**

Ferric ammonium citrate .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution B:****Composition per 100.0mL:**

D-Glucose .....	10.0g
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**Preparation of Solution B:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution C:****Composition per 100.0mL:**

Povidone-iodine .....	0.1g
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**Preparation of Solution C:** Add povidone-iodine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 1.0L of cooled, sterile basal solution, aseptically add 5.0mL of sterile solution A, 10.0mL of sterile solution B, and 10.0mL of sterile solution C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas aeruginosa* and *Pseudomonas (Burkholderia) cepacia*.

**Defined Medium for Rhodopseudomonas****Composition per liter:**

Malic acid .....	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.075g
EDTA .....	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.012g
Thiamine .....	1.0mg
Biotin .....	0.015mg
Trace elements .....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Trace Elements:****Composition per 250.0mL:**

H <sub>3</sub> BO <sub>3</sub> .....	0.7g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.4g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.19g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.06g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.05g
Cu(NO <sub>3</sub> ) <sub>2</sub> ·3H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodobacter capsulatus*.

**Dehalobacter restrictus Medium  
(DSMZ Medium 732)****Composition per 1046mL:**

Solution A .....	900.0mL
Solution B .....	100.0mL
Solution G .....	15.0mL
Solution C .....	10.0mL
Solution E .....	10.0mL
Solution F .....	10.0mL
Solution D .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Solution A:****Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	0.653g
Na-acetate .....	0.460g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	0.173g
Peptone .....	0.1g
Resazurin .....	0.5mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0 L. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature under 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas. Distribute 9 ml volumes into 50 mL serum bottles under 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas. Pressurize closed bottles with H<sub>2</sub> + CO<sub>2</sub> gas to 0.5 bar overpressure. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition per 100mL:**

NaHCO <sub>3</sub> .....	3.730g
NH <sub>4</sub> HCO <sub>3</sub> .....	0.443g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 100.0 mL in bottles. Mix thoroughly. Flush solution with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas. Close bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition per 10mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.11g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0 mL in bottles. Mix thoroughly. Flush solution with 100% N<sub>2</sub> for 20 min. Close bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition per 10mL:**

Na <sub>2</sub> -EDTA .....	5.0mg
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.0mg
AlCl <sub>3</sub> .....	0.1mg
Trace elements solution SL-10 .....	10.0mL

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Solution D:** Add Na<sub>2</sub>-EDTA, FeCl<sub>2</sub>·4H<sub>2</sub>O, and AlCl<sub>3</sub> to 10.0 mL trace solution SL-10 in a Hungate bottle. Mix thoroughly. Flush solution with 100% N<sub>2</sub> for 20 min. Close bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E:**

**Composition** per liter:

Vitamin solution .....	900.0mL
Seven vitamin solution .....	100.0mL

**Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Seven Vitamin Solution:**

**Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
p-Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly.

**Preparation of Solution E:** Combine 900.0mL vitamin solution and 100.0mL seven vitamin solution. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution F:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution G:**

Hexadecane .....	45.0mL
Tetrachloroethene .....	5.0mL

**Preparation of Solution G:** Using a syringe, aseptically inject 5.0mL sterile tetrachloroethene to the 45.0mL sterile hexadecane in the 100mL serum bottle. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Hexadecane:**

Hexadecane .....	45.0mL
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**Preparation of Hexadecane:** Add hexadecane to a 100mL serum bottle. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Tetrachloroethene:**

Tetrachloroethene .....	10.0mL
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**Preparation of Tetrachloroethene:** Add tetrachloroethene to a 10mL serum bottle. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add 9mL sterile solution A to a 50 mL sterile bottle. Then add by injection 1.0mL sterile solution B, 0.1mL sterile solution C, 0.01mL sterile solution D, 0.1mL sterile solution E, and 0.1mL sterile solution F. Inoculate the culture into the medium. Then add by injection 0.15mL sterile solution G.

**Use:** For the cultivation of *Dehalobacter restrictus* and *Sulfurospirillum halorespiran*.

***Deleya halophila* Medium**

**Composition** per liter:

NaCl .....	81.0g
MgSO <sub>4</sub> .....	19.6g
Yeast extract .....	10.0g
Proteose peptone No.3 .....	5.0g
MnCl <sub>2</sub> .....	4.0g
KCl .....	2.0g
Glucose .....	1.0g
CaCl <sub>2</sub> .....	0.47g
NaBr .....	0.026g
NaHCO <sub>3</sub> solution .....	10.0mL
pH 7.5 ± 0.2 at 25°C	

**NaHCO<sub>3</sub> Solution**

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	0.06g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Deleya halophila*.

**Demi-Fraser Broth**

**Composition** per liter:

NaCl .....	20.0g
Tryptose .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	9.6g
Beef extract .....	5.0g
Yeast extract .....	5.0g
LiCl .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.35g
Esculin .....	1.0g
Acridine-HCl .....	12.5mg
Nalidixic acid .....	10.0mg
Ferric ammonium citrate supplement .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder and supplement from BD Diagnostic Systems.

**Ferric Ammonium Citrate Supplement:****Composition per 10.0mL:**

Ferric ammonium citrate.....0.5g

**Preparation of Ferric Ammonium Citrate Supplement:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ferric ammonium citrate supplement, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile ferric ammonium citrate supplement. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Listeria* species from environmental samples.

***Denitrovibrio* Medium  
(DSMZ Medium 881)**

**Composition per 1032mL:**

NaCl.....20.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O.....3.0g  
KH<sub>2</sub>PO<sub>4</sub>.....1.0g  
NaNO<sub>3</sub>.....0.7g  
KCl.....0.5g  
NH<sub>4</sub>Cl.....0.25g  
CaCl<sub>2</sub>·2H<sub>2</sub>O.....0.15g  
Na<sub>2</sub>SO<sub>4</sub>.....0.02g  
Resazurin.....0.5mg  
Na-acetate solution.....10.0mL  
NaHCO<sub>3</sub> solution.....10.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution.....10.0mL  
Seven vitamin solution.....1.0mL  
Trace elements solution SL-10.....1.0mL  
pH 6.8-7.2 at 25°C

**Na-Acetate Solution:****Composition per 10.0mL:**

Na-acetate.....1.64g

**Preparation of Na-Acetate Solution:** Add Na-acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O.....0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO<sub>3</sub>.....2.5g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Seven Vitamin Solution:****Composition per liter:**

Pyridoxine hydrochloride.....300.0mg  
Thiamine-HCl·2H<sub>2</sub>O.....200.0mg  
Nicotinic acid.....200.0mg  
Vitamin B<sub>12</sub>.....100.0mg  
Calcium pantothenate.....100.0mg

*p*-Aminobenzoic acid.....80.0mg  
D(+)-Biotin.....20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O.....1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O.....190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O.....100.0mg  
ZnCl<sub>2</sub>.....70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O.....36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O.....24.0mg  
H<sub>3</sub>BO<sub>3</sub>.....6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O.....2.0mg  
HCl (25% solution).....10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, Na-acetate solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, seven vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 949.0mL. Mix thoroughly. Adjust pH to 6.8-7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL Na-acetate solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 1.0mL seven vitamin solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Denitrovibrio acetiphilus*.

**Deoxycholate Agar**

**Composition per liter:**

Agar.....16.0g  
Lactose.....10.0g  
NaCl.....5.0g  
Pancreatic digest of casein.....5.0g  
Peptic digest of animal tissue.....5.0g  
K<sub>2</sub>HPO<sub>4</sub>.....2.0g  
Ferric citrate.....1.0g  
Sodium citrate.....1.0g  
Sodium deoxycholate.....1.0g  
Neutral Red.....0.033g  
pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 45°–50°C. Pour into sterile Petri dishes.

**Use:** For the selective isolation, cultivation, enumeration, and differentiation of Gram-negative enteric microorganisms from a variety of clinical and nonclinical specimens. *Escherichia coli* appears as large, flat, rose-red colonies. *Enterobacter* and *Klebsiella* species appear as large, mucoid, pale colonies with a pink center. *Proteus* and *Salmonella* species appear as large, colorless to tan colonies. *Shigella* species appear as colorless to pink colonies.

*Pseudomonas* species appear as irregular colorless to brown colonies.

### Deoxycholate Agar (Desoxycholate Agar)

**Composition** per liter:

Agar .....	15.0g
Lactose .....	10.0g
Peptone.....	10.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Ferric citrate.....	1.0g
Sodium citrate.....	1.0g
Sodium deoxycholate.....	1.0g
Neutral Red.....	0.03g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath and BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 50°C. Pour into sterile Petri dishes.

**Use:** For the selective isolation, cultivation, enumeration, and differentiation of Gram-negative enteric microorganisms from a variety of clinical and nonclinical specimens. *Escherichia coli* appears as large, flat, rose-red colonies. *Enterobacter* and *Klebsiella* species appear as large, mucoid, pale colonies with a pink center. *Proteus* and *Salmonella* species appear as large, colorless to tan colonies. *Shigella* species appear as colorless to pink colonies. *Pseudomonas* species appear as irregular colorless to brown colonies.

### Deoxycholate Citrate Agar

**Composition** per liter:

Sodium citrate .....	50.0g
Agar .....	15.0g
Lactose .....	10.0g
Beef extract.....	5.0g
Peptone.....	5.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	5.0g
Sodium deoxycholate.....	2.5g
Ferric citrate.....	1.0g
Neutral Red.....	0.025g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 45°–50°C. Pour into sterile Petri dishes. Dry the agar surface before use.

**Use:** For the selective isolation and cultivation of enteric pathogens, especially *Salmonella* and *Shigella* species.

### Dermabacter Medium

**Composition** per liter:

Pancreatic digest of casein.....	10.0g
Glucose .....	5.0g
NaCl .....	5.0g
Yeast extract.....	5.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Dermabacter hominus*.

### Desulfacinum hydrothermale Medium (DSMZ Medium 875)

**Composition** per 1004mL:

Solution A .....	920.0mL
Solution C (NaHCO <sub>3</sub> solution).....	50.0mL
Solution F .....	13.0mL
Solution D (Seven vitamin solution).....	10.0mL
Solution E.....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL

pH 7.0-7.3 at 25°C

**Solution A:**

**Composition** per 920mL:

NaCl .....	10.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.72g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.24g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.56g
KCl .....	0.29g
NH <sub>4</sub> Cl.....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	0.08g
Resazurin.....	0.5mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C (NaHCO<sub>3</sub> Solution):**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C (NaHCO<sub>3</sub> Solution):**

Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D (Seven Vitamin Solution):**

**Composition** per liter:

Pyridoxine hydrochloride.....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate.....	100.0mg
p-Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Solution D (Seven Vitamin Solution):** Add components to distilled/deionized water and

bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

#### Solution E:

##### Composition per 10.0mL:

Na-lactate ..... 2.5g

**Preparation of Solution E:** Add Na-lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

##### Composition per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 3.0g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add 50.0mL sterile solution C, 13.0mL sterile solution F, 10.0mL sterile solution D, 10.0mL sterile solution E, and 1.0mL sterile solution B, to 920.0mL sterile solution A. Mix thoroughly. The pH of the completed medium should be 7.0–7.3. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Desulfacinum hydrothermale*.

### *Desulfitobacterium dehalogenans* Medium

#### Composition per liter:

Solution A ..... 955.0mL

Solution B ..... 25.0mL

Solution C ..... 20.0mL

#### Solution A:

##### Composition per 955.0mL:

Na<sub>2</sub>HPO<sub>4</sub> ..... 2.2g

Yeast extract ..... 2.0g

3-Chloro-4-hydroxy-phenylacetic acid ..... 1.5g

L-Cysteine-HCl·H<sub>2</sub>O ..... 0.7g

NH<sub>4</sub>Cl ..... 0.5g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.44g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g

CaCl<sub>2</sub> ..... 25.0mg

Wolfe's mineral solution ..... 10.0mL

#### Wolfe's Mineral Solution:

##### Composition per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g

Nitrilotriacetic acid ..... 1.5g

NaCl ..... 1.0g

MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.5g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g

CaCl<sub>2</sub> ..... 0.1g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g

AlK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.01g

H<sub>3</sub>BO<sub>3</sub> ..... 0.01g

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L.

**Preparation of Solution A:** Add components, except L-cysteine-HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 955.0mL. Mix thoroughly. Gently heat and bring

to boiling. Cool to room temperature while sparging with 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Adjust pH to 7.3. Add L-cysteine-HCl·H<sub>2</sub>O. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B:

##### Composition per 25.0mL:

Sodium pyruvate ..... 2.2g

**Preparation of Solution B:** Add sodium pyruvate to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### Solution C:

##### Composition per 20.0mL:

NaHCO<sub>3</sub> ..... 1.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% CO<sub>2</sub>.

**Preparation of Medium:** Aseptically and anaerobically combine 955.0mL of sterile solution A with 25.0mL of sterile solution B and 20.0mL of sterile solution C. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfitobacterium dehalogenans*.

### *Desulfitobacterium hafniense* Medium

#### Composition per 1005.0mL:

NaHCO<sub>3</sub> ..... 2.6g

NH<sub>4</sub>Cl ..... 1.0g

Yeast extract ..... 1.0g

K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O ..... 0.4g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g

NaCl ..... 0.1g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g

Resazurin ..... 0.5mg

Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL

Sodium pyruvate solution ..... 10.0mL

Wolfe's vitamin solution ..... 10.0mL

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution ..... 5.0mL

Selenite-tungstate solution ..... 1.0mL

Trace elements solution SL-10 with EDTA ..... 1.0mL

pH 7.5 ± 0.2 at 25°C

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Sodium Pyruvate Solution:

##### Composition per 10.0mL:

Sodium pyruvate ..... 2.5g

**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Wolfe's Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl ..... 10.0mg

p-Aminobenzoic acid ..... 5.0mg

Lipoic acid ..... 5.0mg

Nicotinic acid ..... 5.0mg

Riboflavin ..... 5.0mg

Thiamine-HCl ..... 5.0mg

Calcium DL-pantothenate.....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	2.5g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Selenite-Tungstate Solution:**

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Trace Elements Solution SL-10 with EDTA:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
Disodium EDTA .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10 with EDTA:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub>, Na<sub>2</sub>S·9H<sub>2</sub>O solution, sodium pyruvate solution, vitamin solution, and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Adjust pH to 7.0. Anaerobically distribute 9.7mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 0.1mL of sterile sodium pyruvate solution, 0.1mL of sterile vitamin solution, and 0.05mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Desulfitobacterium hafniense*.

#### ***Desulfitobacterium Medium* (DSMZ Medium 663)**

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	5.44g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.032g

Resazurin .....	0.5mg
Vitamin solution .....	20.0mL
Na-pyruvate solution .....	10.0mL
Na-thiosulfate solution .....	10.0mL
Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution .....	5.0mL

pH 7.5 ± 0.2 at 25°C

#### **Na-pyruvate Solution:**

**Composition** per 10.0mL:

Na-pyruvate .....	2.5g
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**Preparation of Na-pyruvate Solution:** Add Na-pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### **Na-thiosulfate Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	2.5g
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**Preparation of Na-thiosulfate Solution:** Add 2.5g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Cysteine Solution:**

**Composition** per 10.0mL:

L-Cysteine·HCl·H <sub>2</sub> O .....	0.4g
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**Preparation of Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Elements Solution:**

**Composition** per liter:

Nitritotriacetic acid.....	12.8g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.35g
NaCl .....	1.0g
CoCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.24g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.026g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly. Adjust pH to 6.8.

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg

<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub>. Add components, except vitamin solution, cysteine solution, Na-pyruvate solution, Na-thiosulfate solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 20.0mL sterile vitamin solution, 10.0mL of sterile cysteine solution 10.0mL sterile, Na-pyruvate solution, 10.0mL sterile Na-thiosulfate solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 7.5. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfitobacterium dehalogenans*.

### *Desulfitobacterium* PCE Medium (DSMZ Medium 717)

**Composition** per liter:

(NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> .....	2.88g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Yeast extract .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O.....	0.05g
Resazurin .....	0.1mg
NaHCO <sub>3</sub> solution .....	50.0mL
KOH solution.....	20.0mL
Na-lactate .....	20.0mL
Na-fumarate .....	20.0mL
Vitamin solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	3.3mL
Seven vitamin solution.....	1.0mL
Trace elements solution .....	1.0mL
Selenite-tungstate solution .....	1.0mL

pH 7.1 ± 0.2 at 25°C

### Selenite-Tungstate Solution

**Composition** per liter:

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### NaHCO<sub>3</sub> Solution:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

### KOH Solution:

**Composition** per 100.0mL:

KOH .....	10.0g
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**Preparation of KOH Solution:** Add KOH to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

### Seven Vitamin Solution:

**Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

### Na-lactate Solution:

**Composition** per 100.0mL:

Na-lactate .....	25.0g
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**Preparation of Na-lactate Solution:** Add Na-lactate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.



**Na-fumarate Solution:****Composition** per 100.0mL:

Na-fumarate ..... 16.0g

**Preparation of Na-fumarate Solution:** Add Na-fumarate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, KOH solution, Na-lactate solution, Na-fumarate solution, vitamin solution, seven vitamin solution, selenite-tungstate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 873.7mL. Mix thoroughly. Adjust pH to 7.0–7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL NaHCO<sub>3</sub> solution, 3.3mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 20.0mL KOH solution, 20.0mL Na-lactate solution, 20.0mL Na-fumarate solution, 10.0mL vitamin solution, 1.0mL seven vitamin solution, 1.0mL selenite-tungstate solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Desulfitobacterium* sp. and *Desulfitobacterium hafniense*.

***Desulfobacca* Medium  
(DSMZ Medium 728)**

**Composition** per liter:

NaHCO <sub>3</sub> .....	4.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
Na-acetate.....	1.64g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	0.53g
KH <sub>2</sub> PO <sub>4</sub> .....	0.41g
NH <sub>4</sub> Cl.....	0.3g
NaCl.....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.11g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
Resazurin.....	0.5mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
CaCl <sub>2</sub> solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
Selenite-tungstate solution.....	1.0mL
Seven vitamin solution.....	0.2mL

pH 7.1 ± 0.2 at 25°C

**Seven Vitamin Solution:****Composition** per liter:

Pyridoxine hydrochloride.....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	200.0mg
Nicotinic acid.....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate.....	100.0mg
p-Aminobenzoic acid.....	80.0mg
D(+)-Biotin.....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg

NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Selenite-Tungstate Solution****Composition** per liter:

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**CaCl<sub>2</sub> Solution:****Composition** per 10.0mL:CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.11g

**Preparation of CaCl<sub>2</sub> Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, CaCl<sub>2</sub> solution, seven vitamin solution, selenite-tungstate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 977.8mL. Mix thoroughly. Adjust pH to 7.0–7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL CaCl<sub>2</sub> solution, 0.2mL seven vitamin solution, 1.0mL selenite-tungstate solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Desulfobacca acetoxidans*.

***Desulfobacter* Medium**

**Composition** per 1001.0mL:

Solution A.....	870.0mL
Solution C.....	100.0mL
Solution D.....	10.0mL
Solution E (Vitamin solution).....	10.0mL
Solution F.....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL

pH 7.1–7.4 at 25°C

**Solution A:****Composition** per 870.0mL:

NaCl.....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	3.1g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g

KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B (Trace Elements Solution SL-10):**

##### **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

##### **Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Solution D:**

##### **Composition per 10.0mL:**

Sodium acetate·3H <sub>2</sub> O.....	2.5g
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**Preparation of Solution D:** Add sodium acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E (Vitamin Solution):**

##### **Composition per liter:**

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F:**

##### **Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix

thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfobacter* species and *Malonomonas rubra*.

### ***Desulfobacter postgatei* Medium (DSMZ Medium 193)**

##### **Composition per 1001.0mL:**

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
pH 7.1-7.4 at 25°C	

#### **Solution A:**

##### **Composition per 870.0mL:**

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

#### **Solution B (Trace Elements Solution SL-10):**

##### **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

##### **Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

#### **Solution D:**

##### **Composition per 10.0mL:**

Na-acetate·3H <sub>2</sub> O.....	2.5g
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**Preparation of Solution D:** Add Na-acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix

thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution E (Vitamin Solution):

#### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl-2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.10mg

**Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution F:

#### Composition per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, and 10.0mL solution F. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the cultivation of *Desulfobacter postgatei*, *Paracoccus solventivorans*, and *Desulfotomaculum* sp.

### *Desulfobacter sp. Medium* (DSMZ Medium 195)

#### Composition per 991.0mL:

Solution A	870.0mL
Solution C	100.0mL
Solution D	10.0mL
Solution E (Vitamin solution)	10.0mL
Solution B (Trace elements solution SL-10)	1.0mL
pH 7.1–7.4 at 25°C	

### Solution A:

#### Composition per 870.0mL:

NaCl	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.1g
Na <sub>2</sub> SO <sub>4</sub>	3.0g
KCl	0.5g
NH <sub>4</sub> Cl	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

### Solution B (Trace Elements Solution SL-10):

#### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
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CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution C:

#### Composition per 100.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

### Solution D:

#### Composition per 10.0mL:

Na-acetate·3H <sub>2</sub> O	2.5g
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**Preparation of Solution D:** Add Na-acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution E (Vitamin Solution):

#### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl-2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.10mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution F:

#### Composition per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, and 10.0mL solution F. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the cultivation of *Desulfobacter* species.

**Desulfobacterium anilini Medium****Composition per 1011.0mL:**

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution G .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
pH 7.1–7.4 at 25°C	

**Solution A:****Composition per 870.0mL:**

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition per 10.0mL:**

Sodium acetate·3H <sub>2</sub> O .....	2.5g
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**Preparation of Solution D:** Add sodium acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition per liter:**

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg

Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution G:****Composition per 10.0mL:**

Phenol .....	94.0mg
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**Preparation of Solution G:** Add phenol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Prepare solution freshly.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, solution F, and solution G, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Desulfobacterium anilini*.

**Desulfobacterium anilini Medium****Composition per 1002.0mL:**

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution G .....	1.0mL
pH 7.1–7.4 at 25°C	

**Solution A:****Composition per 870.0mL:**

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Solution D:

**Composition** per 10.0mL:

Phenol .....	0.094g
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**Preparation of Solution D:** Add phenol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Filter sterilize.

#### Solution E (Vitamin Solution):

**Composition** per 100.0mL

Nicotinic acid amide .....	35.0mg
Thiamine dichloride .....	30.0mg
<i>p</i> -Aminobenzoic acid .....	20.0mg
Biotin .....	10.0mg
Calcium pantothenate .....	10.0mg
Pyridoxal-HCl .....	10.0mg
Vitamin B <sub>12</sub> .....	5.0mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution G:

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution G:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, solution F, and solution G, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfobacterium anilini*.

### *Desulfobacterium catecholicum* Medium

**Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL

pH 7.1–7.4 at 25°C

#### Solution A:

**Composition** per 870.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Solution D:

**Composition** per 10.0mL:

Sodium benzoate .....	0.37g
Catechol .....	0.055g

**Preparation of Solution D:** Add sodium benzoate and catechol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Filter sterilize.

#### Solution E (Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg

Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfobacterium catecholicum*.

#### *Desulfobacterium cetonicum* Medium

**Composition per 1011.0mL:**

Solution A .....	950.0mL
Solution C .....	10.0mL
Solution D .....	40.0mL
Solution E .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
pH 7.2–7.4 at 25°C	

#### Solution A:

**Composition per 950.0mL:**

NaCl .....	10.0g
Na <sub>2</sub> SO <sub>4</sub> .....	2.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.7g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.3g
NH <sub>4</sub> Cl.....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition per 10.0mL:**

Sodium butyrate .....	1.2g
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**Preparation of Solution C:** Add sodium butyrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution D:

**Composition per 40.0mL:**

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of Solution D:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution E:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically combine 950.0mL of sterile solution A with 1.0mL of sterile solution B, 10.0mL of sterile solution C, 4.0mL of sterile solution D, and 10.0mL of sterile solution E. Mix thoroughly. Check that final pH is 7.2–7.4.

**Use:** For the cultivation of *Desulfobacterium cetonicum*.

#### *Desulfobacterium indolicum* Medium

**Composition per 1002.4mL:**

Solution A .....	900.0mL
Solution C.....	50.0mL
Solution D .....	30.0mL
Solution E (Wolfe's vitamin solution).....	10.0mL
Solution G .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
Solution F .....	1.0mL
Solution H .....	0.4mL
pH 7.6 ± 0.2 at 25°C	

#### Solution A:

**Composition per 900.0mL:**

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
ZnCl <sub>2</sub> .....	0.07g

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution C:

**Composition** per 50.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution D:

**Composition** per 107.7mL:

Indole .....	0.3g
NaCl (30% solution) .....	7.0mL
MgCl <sub>2</sub> ·6H <sub>2</sub> O (40% solution) .....	0.7mL

**Preparation of Solution D:** Prepare and dispense all solutions anaerobically under 100% N<sub>2</sub>. Add indole to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat while stirring until dissolved. Prepare the NaCl solution and the MgCl<sub>2</sub>·6H<sub>2</sub>O solution separately. Autoclave the three solutions separately for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. To 100.0mL of sterile indole solution, aseptically and anaerobically add 7.0mL of sterile NaCl solution and 0.7mL of sterile MgCl<sub>2</sub>·6H<sub>2</sub>O solution. Mix thoroughly.

#### Solution E (Wolfe's Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl .....	0.01g
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Solution E (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution F:

**Composition** per liter:

Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg
NaOH (0.01M solution) .....	1.0L

**Preparation of Solution F:** Add Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O to 1.0L of NaOH solution. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution G:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper.

Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution H:

**Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.5g
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**Preparation of Solution H:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 900.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 50.0mL of sterile solution C, 10.0mL of sterile solution E, 1.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 30.0mL of sterile solution D and 0.4mL of sterile solution H. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfobacterium indolicum*.

### *Desulfobacterium Medium*

**Composition** per 1002.4mL:

Solution A .....	930.0mL
Solution C .....	50.0mL
Solution D (Wolfe's vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution E .....	1.0mL
Solution G .....	0.4mL

pH 7.0 ± 0.2 at 25°C

#### Solution A:

**Composition** per 930.0mL:

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with

distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution C:

**Composition** per 50.0mL:

NaHCO<sub>3</sub> ..... 2.5g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution D (Wolfe's Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 0.01g  
Thiamine-HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Thioctic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 0.1mg

**Preparation of Solution D (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution E:

**Composition** per liter:

Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg  
NaOH (0.01*M* solution) ..... 1.0L

**Preparation of Solution E:** Add Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O to 1.0L of NaOH solution. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution F:

**Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution G:

**Composition** per 10.0mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> ..... 0.5g

**Preparation of Solution G:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 900.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 50.0mL of sterile solution C, 10.0mL of sterile solution D, 1.0mL of sterile solution E, and 10.0mL of sterile solution F. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution G. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfobacterium autotrophicum*.

### *Desulfobacterium* Medium with Lactate

**Composition** per 1002.4mL:

Solution A ..... 930.0mL

Solution C ..... 50.0mL  
Solution D (Wolfe's vitamin solution) ..... 10.0mL  
Solution F ..... 10.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
Solution E ..... 1.0mL  
Solution G ..... 0.4mL

pH 7.0 ± 0.2 at 25°C

#### Solution A:

**Composition** per 930.0mL:

NaCl ..... 21.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.0g  
Na<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
Lactic acid, sodium salt ..... 1.1g  
KCl ..... 0.5g  
NH<sub>4</sub>Cl ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.10g  
ZnCl<sub>2</sub> ..... 0.070g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.036g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.024g  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution C:

**Composition** per 50.0mL:

NaHCO<sub>3</sub> ..... 2.5g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution D (Wolfe's Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 0.01g  
Thiamine-HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Thioctic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 0.1mg

**Preparation of Solution D (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and



bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution E:

**Composition** per liter:

Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg
NaOH (0.01M solution) .....	1.0L

**Preparation of Solution E:** Add Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O to 1.0L of NaOH solution. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution F:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution G:

**Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.5g
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**Preparation of Solution G:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 900.0mL of cooled, sterile solution A, aseptically and anaerobically add, in the following order, 1.0mL of sterile solution B, 50.0mL of sterile solution C, 10.0mL of sterile solution D, 1.0mL of sterile solution E, and 10.0mL of sterile solution F. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution G. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfobacterium autotrophicum*.

### *Desulfobacterium Medium, Modified*

**Composition** per 1002.4mL:

Solution A .....	920.0mL
Solution C .....	50.0mL
Solution D .....	10.0mL
Solution E (Wolfe's vitamin solution) .....	10.0mL
Solution G .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution F .....	1.0mL
Solution H .....	0.4mL

pH 7.0 ± 0.2 at 25°C

#### Solution A:

**Composition** per 920.0mL:

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating

reduction. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution C:

**Composition** per 50.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution D:

**Composition** per 10.0mL:

Sodium acetate·3H <sub>2</sub> O .....	2.5g
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**Preparation of Solution D:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add sodium acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Cap with rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Solution E (Wolfe's Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl .....	0.01g
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Solution E (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution F:

**Composition** per liter:

Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg
NaOH (0.01M solution) .....	1.0L

**Preparation of Solution F:** Add Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O to 1.0L of NaOH solution. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution G:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution G:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Gas under 100%  $\text{N}_2$  for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution H:

**Composition** per 10.0mL:

$\text{Na}_2\text{S}_2\text{O}_4$  ..... 0.5g

**Preparation of Solution H:** Add  $\text{Na}_2\text{S}_2\text{O}_4$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100%  $\text{N}_2$  for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 920.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 50.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, 1.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution H. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfobacter curvatus* and *Desulfobacter latus*.

#### *Desulfobacterium oleovorans* Medium

**Composition** per 1154.0mL:

Solution A ..... 1.0L  
Solution H ..... 67.0mL  
Solution D ..... 50.0mL  
Solution I ..... 13.0mL  
Solution E ..... 10.0mL  
Solution G ..... 10.0mL  
Solution C (Selenite-tungstate solution) ..... 2.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
Solution F ..... 1.0mL

pH 7.2  $\pm$  0.2 at 25°C

#### Solution A:

**Composition** per liter:

$\text{Na}_2\text{SO}_4$  ..... 4.0g  
NaCl ..... 1.0g  
 $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$  ..... 0.4g  
 $\text{NH}_4\text{Cl}$  ..... 0.25g  
 $\text{KH}_2\text{PO}_4$  ..... 0.2g  
 $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$  ..... 0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Continue gassing until pH reaches below 6.0. Seal the flask under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

$\text{FeCl}_2\cdot 4\text{H}_2\text{O}$  ..... 1.5g  
 $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$  ..... 190.0mg  
 $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$  ..... 100.0mg  
 $\text{ZnCl}_2$  ..... 70.0mg  
 $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$  ..... 36.0mg  
 $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$  ..... 24.0mg  
 $\text{H}_3\text{BO}_3$  ..... 6.0mg  
 $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$  ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add  $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution.

Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C (Selenite-Tungstate Solution):

**Composition** per liter:

NaOH ..... 0.5g  
 $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$  ..... 4.0mg  
 $\text{Na}_2\text{SeO}_3\cdot 5\text{H}_2\text{O}$  ..... 3.0mg

**Preparation of Solution C (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution D:

**Composition** per 50.0mL:

$\text{NaHCO}_3$  ..... 2.5g

**Preparation of Solution D:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Gas under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Calcium DL-pantothenate ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin  $\text{B}_{12}$  ..... 0.1mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Filter sterilize.

#### Solution F:

**Composition** per 10.0mL:

Vitamin  $\text{B}_{12}$  ..... 0.5mg

**Preparation of Solution F:** Add Vitamin  $\text{B}_{12}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Filter sterilize.

#### Solution G:

**Composition** per 80.0mL:

Stearic acid ..... 2.85g  
NaOH (4.0M solution) ..... 2.5mL

**Preparation of Solution G:** Add components to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . In a closed bottle, heat in a boiling water bath. Shake until stearic acid dissolves. Autoclave for 15 min at 15 psi pressure–121°C. On storage, solution will solidify and should be remelted before use.

#### Solution H:

**Composition** per liter:

NaCl ..... 286.4g  
 $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$  ..... 44.7g  
 $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$  ..... 2.2g

**Preparation of Solution H:** Add components to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Solution I:****Composition** per 20.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.6g

**Preparation of Solution I:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 1.0L of sterile solution A, add in order: 1.0mL of sterile solution B, 2.0mL of sterile solution C, 50.0mL of sterile solution D, 10.0mL of sterile solution E, 1.0mL of sterile solution F, 10.0mL of sterile solution G, 67.0mL of sterile solution H, and 13.0mL of sterile solution I. Mix thoroughly. Final pH of the medium should be 7.2. Prior to inoculation, add 10.0–20.0mg of sodium dithionate to 1.0L of medium.

**Use:** For the cultivation and maintenance of *Desulfobacterium oleovorans*.

***Desulfobacterium phenolicum* Medium****Composition** per 1002.4mL:

Solution A ..... 930.0mL  
Solution C ..... 50.0mL  
Solution E (Wolfe's vitamin solution) ..... 10.0mL  
Solution G ..... 10.0mL  
Solution D ..... 4.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
Solution F ..... 1.0mL  
Solution H ..... 0.4mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition** per 920.0mL:

NaCl ..... 21.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.0g  
Na<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
KCl ..... 0.5g  
NH<sub>4</sub>Cl ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g  
ZnCl<sub>2</sub> ..... 0.07g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.036g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.024g  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution C:****Composition** per 50.0mL:

NaHCO<sub>3</sub> ..... 2.5g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

**Solution D:****Composition** per 10.0mL:

Sodium benzoate ..... 1.0g  
Phenol ..... 0.1g

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution E (Wolfe's Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl ..... 0.01g  
Thiamine-HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Thioctic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 0.1mg

**Preparation of Solution E (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution F:****Composition** per liter:

Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg  
NaOH (0.01*M* solution) ..... 1.0L

**Preparation of Solution F:** Add Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O to 1.0L of NaOH solution. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution G:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution H:****Composition** per 10.0mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> ..... 0.5g

**Preparation of Solution H:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 920.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 50.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, 1.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Immediately prior to inoculation aseptically and anaerobically add 0.4mL of sterile solution H. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfobacterium phenolicum*.

***Desulfobacula toluolica* Medium  
(DSMZ Medium 383b)**

**Composition** per 1013.5mL:

Solution A .....	930.0mL
Solution C .....	50.0mL
Solution D .....	10.0mL
Solution E .....	10.0mL
Solution G .....	10.0mL
Solution B .....	1.0mL
Solution F .....	1.0mL
Selenite-tungstate solution .....	1.0mL
Vitamin B <sub>12</sub> solution .....	0.5mL
pH 7.0 at 25°C	

**Solution A:**

**Composition** per 930.0mL:

NaCl .....	21.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B:**

**Composition** per liter:

Na-EDTA .....	5.2g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 7.7mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 6.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated, approximately 20 minutes. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

**Solution D:**

**Composition** per 10.0mL:

Na-benzoate .....	0.4g
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**Preparation of Solution D:** Add Na<sub>2</sub>-benzoate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize. Store anaerobically.

**Solution E:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution F:**

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution F:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution G:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B <sub>12</sub> .....	10.0mg
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**Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize.

**Selenite-Tungstate Solution:**

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add solution B, solution C, solution D, solution E, Vitamin B<sub>12</sub>, solution F, selenite-tungstate solution, and solution G to solution A in that order under N<sub>2</sub> gas. Adjust the pH to 7.0.

**Use:** For the cultivation of *Desulfobacula toluolica*.

***Desulfobulbus* Medium**

**Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
pH 7.1–7.4 at 25°C	

**Solution A:****Composition** per 870.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl.....	1.0g
KCl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition** per 10.0mL:

Sodium propionate.....	2.5g
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**Preparation of Solution D:** Add sodium propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition** per liter:

Pyridoxine·HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine·HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine 870.0mL of sterile solution A with 1.0mL of sterile solution B, 100.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, and 10.0mL of sterile solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfobulbus* species.

***Desulfobulbus* Medium****Composition** per 1001.0mL:

Solution A.....	870.0mL
Solution C.....	100.0mL
Solution D.....	10.0mL
Solution E (Vitamin solution).....	10.0mL
Solution F.....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
pH 7.1–7.4 at 25°C	

**Solution A:****Composition** per 870.0mL:

NaCl.....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.3g
KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ .

#### Solution D:

**Composition** per 10.0mL:

Sodium propionate ..... 1.5g

**Preparation of Solution D:** Add sodium propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Calcium DL-pantothenate ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin  $\text{B}_{12}$  ..... 0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition** per 10.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  ..... 0.4g

**Preparation of Solution F:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ .

**Use:** For the cultivation and maintenance of *Desulfobulbus* species and *Streptomyces* species.

### *Desulfobulbus* sp. Medium

#### (DSMZ Medium 196)

**Composition** per 1001.0mL:

Solution A ..... 870.0mL  
Solution C ..... 100.0mL  
Solution D ..... 10.0mL  
Solution E (Vitamin solution) ..... 10.0mL  
Solution F ..... 10.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
pH 7.1–7.4 at 25°C

#### Solution A:

**Composition** per 870.0mL:

$\text{NaCl}$  ..... 21.0g  
 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 3.1g  
 $\text{Na}_2\text{SO}_4$  ..... 3.0g  
 $\text{KH}_2\text{PO}_4$  ..... 0.2g  
 $\text{NH}_4\text{Cl}$  ..... 0.3g  
 $\text{KCl}$  ..... 0.5g  
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 0.15g  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 1.5g  
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 190.0mg  
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 100.0mg  
 $\text{ZnCl}_2$  ..... 70.0mg  
 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 36.0mg  
 $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 24.0mg  
 $\text{H}_3\text{BO}_3$  ..... 6.0mg  
 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

#### Preparation of Solution B (Trace Elements Solution SL-10):

Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

$\text{NaHCO}_3$  ..... 5.0g

**Preparation of Solution C:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  to remove dissolved oxygen.

#### Solution D:

**Composition** per 10.0mL:

Na-propionate ..... 1.5g

**Preparation of Solution D:** Add Na-propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl- $2\text{H}_2\text{O}$  ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin  $\text{B}_{12}$  ..... 0.10mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition** per 10.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  ..... 0.4g

**Preparation of Solution F:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solu-

tion D, 10.0mL solution E, and 10.0mL solution F. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. Addition of 10-20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the cultivation of *Desulfosarcina variabilis*.

### ***Desulfocapsa sulfoexigens* Medium (DSMZ Medium 195b)**

**Composition** per 1001.0mL:

Solution A .....	890.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### **Solution A:**

**Composition** per 890.0mL:

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.1g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.7g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Dissolve 2.7g FeCl<sub>3</sub>·6H<sub>2</sub>O in 890 ml distilled/deionized water. Adjust pH to 7 with 1N NaOH. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

#### **Solution D:**

**Composition** per 10.0mL:

Na-thiosulfate .....	5.0g
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**Preparation of Solution D:** Add Na-thiosulfate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to

room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, and 10.0mL solution D. Adjust pH to 7.2 with sodium bicarbonate or sodium carbonate. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. Alternately distribute solution A to tubes prior to autoclaving. Dispense 8.9 mL amounts under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into Hungate tubes. Seal and autoclave or 15 min at 15 psi pressure–121°C. Before use aseptically add appropriate amounts of remaining solutions to each tube from sterile anaerobic solutions.

**Use:** For the cultivation of *Desulfocapsa sulfoexigens*.

### ***Desulfococcus amylolyticus* Medium**

**Composition** per 1011.0mL:

Sulfur, powdered .....	10.0g
Starch .....	5.0g
NaHCO <sub>3</sub> .....	0.8g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.7g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
NaCl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.44g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
NH <sub>4</sub> Cl .....	0.33g
Yeast extract .....	0.2g
Resazurin .....	1.0mg
Vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 6.2–6.4 at 25°C	

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Elements Solution SL-10:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub> to 10.0mL of HCl. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Desulfurococcus amylolyticus*.

### **Desulfococcus Medium**

**Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin Solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace Elements Solution SL-10) .....	1.0mL
pH 7.1–7.4 at 25°C	

### **Solution A:**

**Composition** per 870.0mL:

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0μg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

### **Solution D:**

**Composition** per 10.0mL:

Sodium benzoate .....	0.6g
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**Preparation of Solution D:** Add sodium benzoate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Solution E (Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Solution F:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfococcus multivorans*.

### **Desulfococcus multivorans Medium**

**Composition** per liter:

NaCl .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
CaSO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Sodium lactate (70% solution) .....	3.5mL

**Preparation of Medium:** Add components, except FeSO<sub>4</sub>·7H<sub>2</sub>O, ascorbic acid, and thioglycollic acid, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 10–15 min. Add FeSO<sub>4</sub>·7H<sub>2</sub>O, ascorbic acid, and thioglycollic acid. Mix thoroughly. Continue to sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> and adjust pH to 7.4. Anaerobically distribute into tubes or flasks. Autoclave for 10 min at 10 psi pressure–115°C.

**Use:** For the cultivation of *Desulfococcus multivorans*.

### **Desulfococcus niacini Medium**

**Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
pH 7.4 ± 0.2 at 25°C	

### **Solution A:**

**Composition** per 870.0mL:

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g



MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.3g
KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0μg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution C:**

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Solution D:**

**Composition per 10.0mL:**

Sodium nicotinate.....	0.82g
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**Preparation of Solution D:** Add sodium nicotinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution E (Vitamin Solution):**

**Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution F:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Adjust pH to 7.4. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfococcus niacini*.

### *Desulfohalobium Medium*

**Composition per 1010.0mL:**

NaCl.....	100.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	20.0g
KCl.....	4.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.7g
NH <sub>4</sub> Cl.....	1.0g
Sodium acetate.....	1.0g
Trypticase.....	1.0g
Yeast extract.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
Sodium (L)-lactate.....	2.5g
Resazurin.....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0μg
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.0 ± 0.2 at 25°C	

#### **Trace Elements Solution SL-10:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	1.0mg
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C. Aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Methanohalophilus oregonense*.

**Desulfomicrobium WHB Medium****Composition** per 1003.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution G .....	1.0mL
Solution H .....	1.0mL

pH 7.1–7.4 at 25°C

**Solution A:****Composition** per 870.0mL:

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.1g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition** per 10.0mL:

Sodium lactate .....	4.0g
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**Preparation of Solution D:** Add sodium lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg

p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution F:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution G (Selenite-Tungstate Solution):****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution G (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution H (Seven Vitamin):****Composition** per liter:

Pyridoxine-HCl .....	0.3g
Thiamine-HCl .....	0.2g
Nicotinic acid .....	0.2g
Calcium DL-pantothenate .....	0.1g
Vitamin B <sub>12</sub> .....	0.1g
p-Aminobenzoic acid .....	80.0mg
Biotin .....	20.0mg

**Preparation of Solution H (Seven Vitamin):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, solution F, solution G, and solution H, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Desulfomicrobium* species.

**Desulfomonile Medium****Composition** per 1002.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution G .....	1.0mL

pH 6.8–7.0 at 25°C

**Solution A:****Composition** per 870.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g

KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Solution D:**

**Composition** per 10.0mL:

Sodium pyruvate.....	4.0g
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**Preparation of Solution D:** Add sodium propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E (Vitamin Solution):**

**Composition** per liter:

Nicotinamide.....	50.0mg
1,4-Naphthoquinone .....	20.0mg
Pyridoxine-HCl .....	5.0mg
Calcium DL-pantothenate.....	5.0mg
Thioctic acid .....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	5.0mg
Folic acid.....	5.0mg
Vitamin B <sub>12</sub> .....	5.0mg
Hemin.....	5.0mg

**Preparation of Solution E (Vitamin Solution):** Add 1,4-naphthoquinone and hemin to 10.0mL of 0.1N NaOH. Mix thoroughly. Add remaining components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution G:**

**Composition** per 10.0mL:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution G:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine 870.0mL of sterile solution A with 1.0mL of sterile solution B, 100.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, and 10.0mL of sterile solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 100% N<sub>2</sub>. Add 50.0mg/L of sodium dithionite prior to inoculation.

**Use:** For the cultivation and maintenance of *Desulfomonile tiedjei*.

### *Desulfomonile tiedjei* Medium

**Composition** per liter:

NaHCO <sub>3</sub> .....	3.0g
PIPES (Piperazine- <i>N,N'</i> -bis-2-ethanesulfonic acid) buffer .....	1.5g
Na <sub>2</sub> SO <sub>4</sub> .....	1.42g
Yeast extract .....	1.0g
Mineral solution .....	20.0mL
Trace metal solution .....	10.0mL
Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> solution .....	10.0mL
Vitamin solution .....	10.0mL
Sodium pyruvate solution.....	10.0mL
Resazurin (0.1% solution) .....	1.0mL
pH 7.3 ± 0.2 at 25°C	

#### **Mineral Solution:**

**Composition** per liter:

NH <sub>4</sub> Cl .....	50.0g
NaCl .....	40.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	8.3g
KCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Trace Metal Solution:**

**Composition** per liter:

Nitrilotriacetic acid.....	2.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.8g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> SeO <sub>4</sub> .....	0.02g
Na <sub>2</sub> WO <sub>4</sub> .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Trace Metal Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Vitamin Solution:****Composition per liter:**

Nicotinamide.....	0.05g
1,4-Naphthoquinone .....	0.02g
<i>p</i> -Aminobenzoic acid .....	5.0mg
Biotin .....	5.0mg
Calcium pantothenate .....	5.0mg
Cyanocobalamin .....	5.0mg
Folic acid.....	5.0mg
Hemin.....	5.0mg
Pyridoxine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Thioctic acid .....	5.0mg
NaOH (0.1 <i>N</i> solution).....	5.0mL

**Preparation of Vitamin Solution:** Add thioctic acid, 1,4-naphthoquinone, and hemin to 5.0mL of 0.1*N* NaOH solution. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Add remaining components. Mix thoroughly.

**Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.087g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Filter sterilize. Prepare freshly.

**Sodium Pyruvate Solution:****Composition per 10.0mL:**

Sodium pyruvate .....	4.4g
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**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 10.0mL. Filter sterilize.

**Preparation of Medium:** Add PIPES buffer, Na<sub>2</sub>SO<sub>4</sub>, yeast extract, mineral solution, and trace metal solution to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 7.3 with HCl. Add NaHCO<sub>3</sub> and resazurin. Gently heat and bring to boiling under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Replace headspace with 2 atm pressure of the same gas phase. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Anaerobically and aseptically add sterile vitamin solution, sodium pyruvate solution, and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Desulfomonile tiedjei*.

***Desulfomusa hansenii* Medium  
(DSMZ Medium 916)**

**Composition per 992.0mL:**

Solution A .....	870.0mL
Solution D .....	50.0mL
Solution F .....	50.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution G .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
Solution C (Selenite-tungstate solution).....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Solution A:****Composition per 870mL:**

NaCl .....	20.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.42g
KCl.....	0.67g
NH <sub>4</sub> Cl.....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	0.01g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
Resazurin .....	0.5mg

**Preparation of Solution A:** Add components to 870.0mL with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Solution B (Trace Elements Solution SL-10):****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C (Selenite-Tungstate Solution):****Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution C (Selenite-Tungstate Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition per 50.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution D:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid.....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution F:****Composition per 50mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.52g

**Preparation of Solution F:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution G:****Composition** per 10.0mL:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.52g

**Preparation of Solution G:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Adjust pH to 2.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution H:****Composition** per 10.0mL:

Na-propionate ..... 0.96g

**Preparation of Solution H:** Add Na-propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> sequentially add to 870.0mL sterile solution A, 1.0mL solution B, 1.0mL solution C, 50.0mL solution D, 10.0mL solution E, 50.0mL solution F, and 10.0mL solution G. Aseptically and anaerobically distribute under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. The final pH should be 7.2. Addition of 10-20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the cultivation of *Desulfomusa hansenii*.

***Desulfonatronovibrio* Medium  
(DSMZ Medium 742)**

**Composition** per liter:

NaHCO<sub>3</sub> ..... 15.0g  
Na<sub>2</sub>CO<sub>3</sub> ..... 10.0g  
NaCl ..... 10.0g  
Na<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
NH<sub>4</sub>Cl ..... 1.0g  
Na<sub>2</sub>HPO<sub>4</sub> ..... 0.2g  
KCl ..... 0.2g  
Resazurin ..... 0.5mg  
Yeast extract solution ..... 10.0mL  
Na-formate solution ..... 10.0mL  
Trace elements solution ..... 10.0mL  
Vitamin solution ..... 10.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL

pH 9.6 ± 0.2 at 25°C

**Na-Formate Solution:****Composition** per 10.0mL:

Na-formate ..... 5.0g

**Preparation of Na-Formate Solution:** Add Na-formate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Yeast Extract Solution:****Composition** per 10.0mL:

Yeast extract ..... 1.5g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 1.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL.

Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Trace Elements Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitrilotriacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O ..... 0.01g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub>. Add components, except vitamin solution, yeast extract solution, Na-formate solution, trace elements solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL sterile vitamin solution, 10.0mL of sterile yeast extract solution, 10.0mL sterile Na-formate solution, 10.0mL sterile trace elements solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 9.6. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfonatronovibrio hydrogenovorans*.

***Desulfonatronum* Medium  
(DSMZ Medium 813)**

**Composition** per 1010mL:

NaCl ..... 10.0g  
Na<sub>2</sub>SO<sub>4</sub> ..... 5.0g  
Na<sub>2</sub>CO<sub>3</sub> ..... 3.5g  
NH<sub>4</sub>Cl ..... 1.0g  
Yeast extract ..... 1.0g  
Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
KCl ..... 0.2g

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	0.5mg
Na-formate solution .....	10.0mL
Vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 8.9 ± 0.2 at 25°C	

**Na-Formate Solution:****Composition per 10.0mL:**

Na-formate .....	4.0g
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**Preparation of Na-Formate Solution:** Add Na-formate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub>. Add components, except Na-formate solution and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 30 min. Add the Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Adjust pH to 8.8–9.0. Dispense into Hungate tubes under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add Na-formate solution, 0.1mL per 10.0mL medium.

**Use:** For the cultivation of *Desulfonatronum lacustre*.

### ***Desulfonema ishimotoi* Medium (DSMZ Medium 739)**

**Composition per 957.6mL:**

Solution A .....	850.0mL
Agar solution .....	50.0mL
Solution C .....	50.0mL
Solution H .....	20.0mL
Solution D .....	10.0mL

Solution F (Vitamin solution) .....	10.0mL
Solution I .....	10.0mL
Solution G (Artificial sediment) .....	6.6mL
Solution E .....	1.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
pH 6.9 ± 0.2 at 25°C	

**Solution A:****Composition per 800.0mL:**

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.5g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.35g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Resazurin .....	0.5mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3μg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly.

**Solution B (Trace Elements Solution SL-10):****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

**Solution D:****Composition per 10.0mL:**

Na-acetate·3H <sub>2</sub> O .....	2.5g
Isobutyric acid .....	0.18g
Na <sub>2</sub> -succinate .....	0.1g

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution E:****Composition per 10.0mL:**

Na <sub>2</sub> -succinate .....	1.0g
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**Preparation of Solution E:** Add Na<sub>2</sub>-succinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F (Vitamin Solution):****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Vitamin B <sub>12</sub> .....	5.1mg

Thiamine-HCl-2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg

**Preparation of Solution F (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Solution G (Artificial Sediment):**

##### **Composition** per 6.6mL:

AlCl <sub>3</sub> ·6H <sub>2</sub> O, 4.9% (w/v) .....	5.0mL
Na <sub>2</sub> CO <sub>3</sub> , 10.6% (w/v) .....	1.6mL

**Preparation of Solution G (Artificial Sediment):** Combine components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution H:**

##### **Composition** per 20.0mL:

Rumen fluid, clarified .....	20.0mL
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**Preparation of Solution H:** Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution I:**

##### **Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution I:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Add 50.0mL hot agar solution. Mix thoroughly. Cool to 50°C. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 1.0mL solution E, 10.0mL solution F, 6.6mL solution G, 20.0mL solution H, and 10.0mL solution I. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. The pH should be 6.9. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the cultivation of *Desulfonema ishimotonii*.

### ***Desulfonema limicola* Medium (DSMZ Medium 201)**

##### **Composition** per 1007.6mL:

Solution A .....	850.0mL
Solution C .....	100.0mL
Solution H .....	20.0mL
Solution D .....	10.0mL
Solution F (Vitamin solution) .....	10.0mL
Solution I .....	10.0mL
Solution G (Artificial sediment) .....	6.6mL
Solution E .....	1.0mL
Solution B (Trace Elements Solution SL-10) .....	1.0mL
pH 7.6 ± 0.2 at 25°C	

#### **Solution A:**

##### **Composition** per 870.0mL:

NaCl .....	13.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.2g
KCl .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
NH <sub>4</sub> Cl .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

#### **Solution B (Trace Elements Solution SL-10):**

##### **Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

##### **Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

#### **Solution D:**

##### **Composition** per 10.0mL:

Na-acetate·3H <sub>2</sub> O .....	2.5g
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**Preparation of Solution D:** Add Na-acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E:**

##### **Composition** per 10.0mL:

Na <sub>2</sub> -succinate .....	1.0g
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**Preparation of Solution E:** Add Na<sub>2</sub>-succinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F (Vitamin Solution):**

##### **Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl-2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.10mg

**Preparation of Solution F (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution G (Artificial Sediment):****Composition** per 6.6mL:

AlCl <sub>3</sub> ·6H <sub>2</sub> O, 4.9% (w/v)	5.0mL
Na <sub>2</sub> CO <sub>3</sub> , 10.6% (w/v)	1.6mL

**Preparation of Solution G (Artificial Sediment):**

Combine components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution H:****Composition** per 20.0mL:

Rumen fluid, clarified	20.0mL
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**Preparation of Solution H:** Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution I:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.4g
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**Preparation of Solution I:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 1.0mL solution E, 10.0mL solution F, 6.6mL solution G, 20.0mL solution H, and 10.0mL solution I. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. The pH should be 7.6. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the cultivation of *Desulfonema limicola*.

***Desulfonema magnum* Medium  
(DSMZ Medium 202)**

**Composition** per 957.6mL:

Solution A	850.0mL
Solution C	50.0mL
Solution H	20.0mL
Solution D	10.0mL
Solution F (Vitamin solution)	10.0mL
Solution I	10.0mL
Solution G (Artificial sediment)	6.6mL
Solution E	1.0mL
Solution B (Trace elements solution SL-10)	1.0mL
pH 6.9 ± 0.2 at 25°C	

**Solution A:****Composition** per 870.0mL:

NaCl	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	5.5g
Na <sub>2</sub> SO <sub>4</sub>	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.35g
KCl	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
NH <sub>4</sub> Cl	0.3g
Resazurin	0.5mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3μg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

**Solution D:****Composition** per 10.0mL:

Na-acetate	0.6g
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**Preparation of Solution D:** Add Na-acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E:****Composition** per 10.0mL:

Na <sub>2</sub> -succinate	1.0g
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**Preparation of Solution D:** Add Na<sub>2</sub>-succinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl	10.0mg
Vitamin B <sub>12</sub>	5.1mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg

**Preparation of Solution F (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution G (Artificial Sediment):****Composition** per 6.6mL:

AlCl <sub>3</sub> ·6H <sub>2</sub> O, 4.9% (w/v)	5.0mL
Na <sub>2</sub> CO <sub>3</sub> , 10.6% (w/v)	1.6mL

**Preparation of Solution G (Artificial Sediment):**

Combine components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.



**Solution H:****Composition** per 20.0mL:

Rumen fluid, clarified .....20.0mL

**Preparation of Solution H:** Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.**Solution I:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g**Preparation of Solution I:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 1.0mL solution E, 10.0mL solution F, 6.6mL solution G, 20.0mL solution H, and 10.0mL solution I. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. The pH should be 6.9. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.**Use:** For the cultivation of *Desulfonema magnum*.***Desulforhabdus amnigenus* Medium  
(DSMZ Medium 708)****Composition** per liter:

Na <sub>2</sub> SO <sub>4</sub> .....	2.8g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	0.53g
KH <sub>2</sub> PO <sub>4</sub> .....	0.41g
NH <sub>4</sub> Cl .....	0.3g
NaCl .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.11g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	0.5mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Na-propionate solution .....	10.0mL
Selenite-tungstate solution .....	1.0mL
Seven vitamin solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.2–7.6 at 25°C

**Na-propionate Solution:****Composition** per 10.0mL:

Na-propionate .....2.0g

**Preparation of Na-propionate Solution:** Add Na-propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.**Selenite-Tungstate Solution:****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10mL:Na<sub>2</sub>S·9H<sub>2</sub>O .....0.5g**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.**NaHCO<sub>3</sub> Solution:****Composition** per 10.0mL:NaHCO<sub>3</sub> .....4.0g**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.**Seven Vitamin Solution:****Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na-

propionate solution, vitamin solution, seven vitamin solution, selenite-tungstate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 947.0mL. Mix thoroughly. Adjust pH to 7.2-7.6. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL Na-propionate solution, 10.0mL vitamin solution, 1.0mL seven vitamin solution, 1.0mL selenite-tungstate solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Desulforhabdus amnigena* (*Desulforhabdus amnigenus*).

### Desulfosarcina Medium

#### Composition per 1001.0mL:

Solution A	870.0mL
Solution C	100.0mL
Solution D	10.0mL
Solution E (Vitamin solution)	10.0mL
Solution F	10.0mL
Solution B (Trace elements solution SL-10)	1.0mL
pH 7.1-7.4 at 25°C	

#### Solution A:

##### Composition per 870.0mL:

NaCl	7.0g
Na <sub>2</sub> SO <sub>4</sub>	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	1.3g
KCl	0.5g
NH <sub>4</sub> Cl	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0μg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### Solution B (Trace Elements Solution SL-10):

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### Solution C:

##### Composition per 100.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Solution D:

##### Composition per 10.0mL:

Sodium benzoate	0.6g
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**Preparation of Solution D:** Add sodium benzoate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### Solution E (Vitamin Solution):

##### Composition per liter:

Pyridoxine·HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine·HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### Solution F:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfosarcina variabilis*.

### Desulfosarcina variabilis Medium

#### Composition per 1009.0mL:

Solution A	850.0mL
Solution C	100.0mL
Solution G	20.0mL
Solution D	10.0mL
Solution E (Wolfe's vitamin solution)	10.0mL
Solution H	10.0mL
Solution F	6.6mL
Solution B (Trace elements solution SL-10)	1.0mL
Solution I	0.4mL

pH 7.6 ± 0.2 at 25°C

#### Solution A:

##### Composition per 920.0mL:

NaCl	13.5g
Na <sub>2</sub> SO <sub>4</sub>	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.2g
KCl	0.5g
NH <sub>4</sub> Cl	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0μg
Resazurin	0.5mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to

920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction, and a pH of 6.0 is reached. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.10g
ZnCl <sub>2</sub> .....	0.070g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### **Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### **Solution D:**

**Composition** per 10.0mL:

Sodium benzoate .....	0.6g
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**Preparation of Solution D:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add sodium benzoate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution E (Wolfe's Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl .....	0.01g
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Solution E (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### **Solution F:**

**Composition** per 6.6mL:

AlCl <sub>3</sub> ·6H <sub>2</sub> O (4.9% solution) .....	5.0mL
Na <sub>2</sub> CO <sub>3</sub> (10.6% solution) .....	1.6mL

**Preparation of Solution F:** Combine both solutions. Mix thoroughly. Gas with 100% N<sub>2</sub>. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution G:**

**Composition** per 10.0mL:

Rumen fluid, clarified .....	20.0mL
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**Preparation of Solution G:** Gas rumen fluid under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution H:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution H:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution I:**

**Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.5g
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**Preparation of Solution I:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 850.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 1000.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, 6.6mL of sterile solution F, 20.0mL of sterile solution G, and 10.0mL of sterile solution H. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution I. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfosarcina variabilis*.

### *Desulfotomaculum acetoxidans* Medium

**Composition** per 1011.0mL:

Solution A .....	1.0L
Solution B .....	10.0mL
Vitamin solution .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

#### **Solution A:**

**Composition** per liter:

NaHCO <sub>3</sub> .....	4.5g
Na <sub>2</sub> SO <sub>4</sub> .....	2.84g
Sodium acetate .....	1.4g
Sodium butyrate .....	1.4g
NaCl .....	1.17g
Yeast extract .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
KCl .....	0.3g
NH <sub>4</sub> Cl .....	0.27g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg
Trace elements solution .....	1.0mL

**Preparation of Solution A:** Add components, except NaHCO<sub>3</sub> and vitamin solution, and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under O<sub>2</sub>-free 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub> and continue gassing with O<sub>2</sub>-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> until pH reaches 6.9–7.1. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	120.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	68.0mg
H <sub>3</sub> BO <sub>3</sub> .....	62.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	24.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	17.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>.

**Vitamin Solution:****Composition per 100.0mL:**

Thiamine·HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	4.0mg
D(+)-Biotin .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 100% N<sub>2</sub>.

**Solution B:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.36g
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**Preparation of Solution B:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 1.0L of sterile solution A, add 10.0mL of sterile solution B and 1.0mL of sterile vitamin solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Desulfotomaculum acetoxidans*.

***Desulfotomaculum acetoxidans* Medium****Composition per 1020.0mL:**

Solution A .....	1.0L
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL

**Solution A:****Composition per liter:**

NaHCO <sub>3</sub> .....	4.5g
Na <sub>2</sub> SO <sub>4</sub> .....	2.84g
Sodium acetate .....	1.4g
Sodium butyrate .....	1.4g
NaCl .....	1.17g
Yeast extract .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
KCl .....	0.3g
NH <sub>4</sub> Cl .....	0.27g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg
Trace elements solution SL-7 .....	1.0mL

**Trace Elements Solution SL-7:****Composition per 1010.0mL:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
H <sub>3</sub> BO <sub>3</sub> .....	62.0mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	17.0mg
Hydrochloric acid, 25% .....	10.0mL

**Preparation of Trace Elements Solution SL-7:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Continue sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub> until pH stabilizes at 6.9–7.1. Anaerobically distribute into tubes in 10.0mL amounts under an atmosphere of 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Aseptically add 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 0.1mL of sterile Wolfe's vitamin solution to each tube containing 10.0mL of solution A. Prepare immediately prior to use.

**Use:** For the cultivation of *Desulfotomaculum acetoxidans*.

***Desulfotomaculum alkaliphilum* Medium  
(DSMZ Medium 866)****Composition per liter:**

NaHCO <sub>3</sub> .....	8.0g
Na <sub>2</sub> SO <sub>4</sub> .....	5.0g
Na-formate .....	5.0g
NaCl .....	5.0g
Yeast extract .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
KCl .....	0.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 8.7–9.0 at 25°C

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl-2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Desulfotomaculum alkaliphilum*.

***Desulfotomaculum geothermicum* Medium****Composition** per 1001.0mL:

Solution A	870.0mL
Solution C	100.0mL
Solution D	10.0mL
Solution E (Vitamin solution)	10.0mL
Solution F	10.0mL
Solution B (Trace elements solution SL-10)	1.0mL
pH 7.1–7.4 at 25°C	

**Solution A:****Composition** per 870.0mL:

NaCl	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.1g
Na <sub>2</sub> SO <sub>4</sub>	3.0g
KCl	0.5g
NH <sub>4</sub> Cl	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until

pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition** per 10.0mL:

Sodium lactate	2.5g
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**Preparation of Solution D:** Add Na lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Solution E (Vitamin Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.05g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfotomaculum geothermicum*.

**Desulfotomaculum Groll Medium  
(DSMZ Medium 124a)**

**Composition per liter:**

NaHCO <sub>3</sub> .....	4.5g
Na <sub>2</sub> SO <sub>4</sub> .....	2.84g
Na-acetate.....	1.4g
Na-butyrate.....	1.4g
NaCl.....	1.17g
Yeast extract.....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
KCl.....	0.3g
NH <sub>4</sub> Cl.....	0.27g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	0.5mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Substrate solution.....	10.0mL
Selenite solution.....	10.0mL
Vitamin solution.....	1.0mL
Trace elements solution.....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	3.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Trace Elements Solution:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	120.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	68.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	24.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	62.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	17.0mg
HCl (0.5M).....	1.0L

**Preparation of Trace Elements Solution:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 1.0L of 0.5M HCl. Mix thoroughly. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition 100.0mL:**

Thiamine-HCl·2H <sub>2</sub> O.....	10.0mg
p-Aminobenzoic acid.....	4.0mg
D(+)-Biotin.....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Selenite Solution:****Composition per 10.0mL:**

Sodium selenite.....	3.0μg
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**Preparation of Selenite Solution:** Add sodium selenite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Substrate Solution:****Composition per 10.0mL:**

Sodium benzoate.....	0.6g
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**Preparation of Substrate Solution:** Add sodium benzoate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except substrate solution, selenite solution, vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Heat gently and bring to boiling. Cool to room temperature. Add NaHCO<sub>3</sub>. Continue sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub> until an equilibrium pH of 6.9–7.1 is reached. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 10.0mL of sterile substrate solution, 10.0mL of sterile selenite solution, 1.0mL of sterile vitamin solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks. Alternately the medium can be distributed to tubes under anaerobic conditions and autoclaved in tubes prior to addition of substrate solution, selenite solution, vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution. Appropriate amounts of these solutions can then be added to each tube to yield the desired concentrations. Additions are performed aseptically and anaerobically under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture.

**Use:** For the cultivation of *Desulfotomaculum gibsoniae*.

**Desulfotomaculum halophilum Medium  
(DSMZ Medium 815)**

**Composition per liter:**

Iron, powder.....	150.0g
NaCl.....	40.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	8.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	6.0g
Na-lactate.....	3.6g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MOPS.....	3.0g
KCl.....	2.0g
Yeast extract.....	1.0g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
SrCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
Resazurin.....	0.5mg
NaHCO <sub>3</sub> solution.....	50.0mL
Trace elements solution SL-12.....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Trace Elements Solution SL-12:****Composition per liter:**

Na <sub>2</sub> -EDTA.....	5.2g
FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-12:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 6.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition** per 100.0mL:NaHCO<sub>3</sub> ..... 10.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except iron and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 950.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Adjust pH to 6.0. Dispense under 100% N<sub>2</sub> into tubes or bottles containing 1.5g iron per 10.0mL medium. Autoclave for 30 min at 105°C. Cool to 25°C. Aseptically and anaerobically add sterile NaHCO<sub>3</sub> solution, 0.5mL per 10.0mL medium. Final pH is 7.2.

**Use:** For the cultivation of *Desulfotomaculum halophilum* and *Desulfocella halophila*.

***Desulfotomaculum sapomandens*  
Medium**

**Composition** per 1019.7mL:

Solution A ..... 966.0mL  
 Solution B ..... 20.0mL  
 Solution C ..... 10.0mL  
 Solution D ..... 10.0mL  
 Solution F ..... 10.0mL  
 Solution H ..... 1.7mL  
 Solution G ..... 1.0mL  
 Solution E ..... 1.0mL

pH 7.2–7.5 at 25°C

**Solution A:****Composition** per 966.0mL:

Na<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
 NaCl ..... 1.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.4g  
 NH<sub>4</sub>Cl ..... 0.3g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
 Resazurin ..... 1.0mg  
 Trace elements solution SL-10 ..... 1.0mL

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
 HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 20.0mL:NaHCO<sub>3</sub> ..... 1.0g

**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 10.0mL:

Ethanol ..... 1.0mL

**Preparation of Solution C:** Add ethanol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Solution D:****Composition** per 10.0mL:

Sodium benzoate ..... 0.7g

**Preparation of Solution D:** Add sodium benzoate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Solution E:****Composition** per 1.0mL:

Rumen fluid, clarified ..... 1.0mL

**Preparation of Solution E:** Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F:****Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
 Calcium DL-pantothenate ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Thiamine-HCl ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Solution F:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Filter sterilize.

**Solution G:****Composition** per 1.0mL:

Sodium dithionite ..... 0.025g

**Preparation of Solution G:** Add sodium dithionite to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Filter sterilize.

**Solution H:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Solution H:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine in the following order: 966.0mL of sterile solution A with 20.0mL of sterile solution B, 10.0mL of sterile solution C, 10.0mL of sterile solution D, 1.0mL of sterile solution E, 10.0mL of sterile solution F, 1.0mL of sterile solution G, and 1.7mL of sterile solution H. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Desulfotomaculum sapomandens*.

***Desulfotomaculum* sp. Medium I  
(DSMZ Medium 63a)**

**Composition** per liter:

Solution A ..... 980.0mL  
 Solution B ..... 10.0mL  
 Solution C ..... 10.0mL

pH 6.5–7.0 at 25°C

**Solution A:****Composition** per 980.0mL:

Na-pyruvate .....	5.0g
Na-acetate .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly.

**Solution B:****Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
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**Preparation of Solution B:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Solution C:****Composition** per 10.0mL:

Na-thioglycolate .....	0.1g
Ascorbic acid .....	0.1g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Bring solution A to a boil for a few minutes. Cool to room temperature while gassing with oxygen-free N<sub>2</sub> gas. Add solutions B and C. Adjust pH to 6.5-7.0. Immediately distribute under N<sub>2</sub> into anaerobic tubes. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Desulfotomaculum* spp.

***Desulfotomaculum* species Medium II****Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Seven vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
pH 7.1-7.4 at 25°C	

**Solution A:****Composition** per 870.0mL:

NaCl .....	7.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
Na <sub>2</sub> SO <sub>4</sub> .....	0.7g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition** per 10.0mL:

3,4,5-Trimethoxybenzoate .....	0.42g
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**Preparation of Solution D:** Add 3,4,5-trimethoxybenzoic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Solution E (Seven Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	0.3g
Thiamine-HCl .....	0.2g
Nicotinic acid .....	0.2g
Calcium DL-pantothenate .....	0.1g
Vitamin B <sub>12</sub> .....	0.1g
p-Aminobenzoic acid .....	80.0mg
Biotin .....	20.0mg

**Preparation of Solution E (Seven Vitamin Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Solution F:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Desulfotomaculum* species.

***Desulfotomaculum thermosapovorans* Medium****Composition** per 1015.0mL:

Solution A .....	900.0mL
Solution B .....	100.0mL
Solution C .....	10.0mL



Solution D .....	5.0mL
Solution E .....	0.5mL
pH 7.0–7.2 at 25°C	

**Solution A:****Composition** per 900.0mL:

NaCl .....	8.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
Sodium butyrate .....	2.2g
NH <sub>4</sub> Cl .....	1.0g
KCl .....	0.5g
Sodium acetate .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
Yeast extract .....	0.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.003mg
Trace elements solution SL-10 .....	1.5mL

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution B:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per liter:

Pyridoxine·HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Solution D:****Composition** per 5.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 5.0mL. Mix

thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Solution E:****Composition** per 1.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> (sodium dithionite) .....	20.0mg
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**Preparation of Solution E:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare medium anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically combine 900.0mL of sterile solution A, 100.0mL of sterile solution B, 10.0mL of sterile solution C, 5.0mL of sterile solution D, and 0.5 mL of sterile solution E. Mix thoroughly. Adjust pH to 7.0–7.2.

**Use:** For the cultivation of *Desulfotomaculum thermosapovrans*.

### *Desulfovibrio aespoensis* Medium (DSMZ Medium 721)

**Composition** per 1004.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Selenite-tungstate solution .....	2.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Seven vitamin solution .....	1.0mL
pH 7.3–7.5 at 25°C	

**Solution A:****Composition** per 870.0mL:

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  to remove dissolved oxygen.

#### Solution D:

**Composition** per 10.0mL:

Na-lactate ..... 2.5g

**Preparation of Solution D:** Add Na-lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl-2H<sub>2</sub>O ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.10mg

**Solution E Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition** per 10.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  ..... 0.4g

**Preparation of Solution F:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Seven Vitamin Solution:

**Composition** per liter:

Pyridoxine hydrochloride ..... 300.0mg  
Thiamine-HCl-2H<sub>2</sub>O ..... 200.0mg  
Nicotinic acid ..... 200.0mg  
Vitamin B<sub>12</sub> ..... 100.0mg  
Calcium pantothenate ..... 100.0mg  
*p*-Aminobenzoic acid ..... 80.0mg  
D-(+)-Biotin ..... 20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100%  $\text{N}_2$ . Mix thoroughly. Filter sterilize.

#### Selenite-Tungstate Solution:

**Composition** per liter:

NaOH ..... 0.5g  
 $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  ..... 4.0mg  
 $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$  ..... 3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Filter sterilize.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, 10.0mL solution F, 2.0mL selenite-tungstate solution, and 1.0mL seven vitamin solution.

Distribute anaerobically under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  into appropriate vessels.

**Use:** For the cultivation of *Desulfovibrio aespoensis*.

#### *Desulfovibrio alcoholovorans* Medium

**Composition** per liter:

Solution A ..... 980.0mL  
Solution B ..... 10.0mL  
Solution C ..... 10.0mL  
pH 7.8 ± 0.2 at 25°C

#### Solution A:

**Composition** per 980.0mL:

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 2.0g  
1,2-Propanediol ..... 1.5g  
 $\text{Na}_2\text{SO}_4$  ..... 1.0g  
 $\text{NH}_4\text{Cl}$  ..... 1.0g  
Yeast extract ..... 1.0g  
 $\text{K}_2\text{HPO}_4$  ..... 0.5g  
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 0.1g  
 $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$  ..... 3.0mg  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 100%  $\text{N}_2$ .

#### Solution B:

**Composition** per 10.0mL:

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.5g

**Preparation of Solution B:** Add  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 10.0mL:

Ascorbic acid ..... 0.1g  
Sodium thioglycolate ..... 0.1g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 980.0mL of cooled solution A, anaerobically add 10.0mL of solution B and 10.0mL of solution C. Mix thoroughly. Adjust pH to 7.8 with NaOH. Distribute into tubes or flasks. During distribution, swirl the medium to keep the precipitate in suspension. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Desulfovibrio alcoholovorans*.

#### *Desulfovibrio asponium* Medium

**Composition** per 1004.0mL:

Solution A ..... 870.0mL  
Solution C ..... 100.0mL  
Solution D ..... 10.0mL  
Solution E (Vitamin solution) ..... 10.0mL  
Solution F ..... 10.0mL  
Solution G (Selenite-tungstate solution) ..... 2.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
Solution H (Seven vitamin solution) ..... 1.0mL  
pH 7.3–7.5 at 25°C

#### Solution A:

**Composition** per 870.0mL:

NaCl ..... 7.0g

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.3g
KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Solution D:**

**Composition** per 10.0mL:

Sodium lactate.....	2.5g
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**Preparation of Solution D:** Add sodium lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution E (Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution F:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution G (Selenite-Tungstate Solution):**

**Composition** per liter:

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

#### **Preparation of Solution G (Selenite-Tungstate Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution H (Seven Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl.....	0.3g
Thiamine-HCl.....	0.2g
Nicotinic acid.....	0.2g
Calcium DL-pantothenate.....	0.1g
Vitamin B <sub>12</sub> .....	0.1g
p-Aminobenzoic acid.....	80.0mg
Biotin.....	20.0mg

#### **Preparation of Solution H (Seven Vitamin Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Desulfovibrio asponium*.

#### ***Desulfovibrio baarsii* Medium**

**Composition** per 1009.0mL:

Solution A.....	850.0mL
Solution C.....	100.0mL
Solution G.....	20.0mL
Solution D.....	10.0mL
Solution E (Wolfe's vitamin solution).....	10.0mL
Solution H.....	10.0mL
Solution F.....	6.6mL
Solution B (Trace elements solution SL-10).....	1.0mL
Solution I.....	0.4mL

pH 7.6 ± 0.2 at 25°C

#### **Solution A:**

**Composition** per 920.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl.....	1.0g
KCl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	0.5mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction, and a pH of 6.0 is reached. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 25°C.

**Solution B (Trace Elements Solution SL-10):****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.10g
ZnCl <sub>2</sub> .....	0.070g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution C:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

**Solution D:****Composition per 10.0mL:**

Sodium butyrate .....	0.7g
Sodium caproate .....	0.3g
Sodium octanoate .....	0.15g

**Preparation of Solution D:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution E (Wolfe's Vitamin Solution):****Composition per liter:**

Pyridoxine·HCl .....	0.01g
Thiamine·HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Solution E (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution F:****Composition per 6.6mL:**

AlCl <sub>3</sub> ·6H <sub>2</sub> O (4.9% solution) .....	5.0mL
Na <sub>2</sub> CO <sub>3</sub> (10.6% solution) .....	1.6mL

**Preparation of Solution F:** Combine both solutions. Mix thoroughly. Gas with 100% N<sub>2</sub>. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution G:****Composition per 10.0mL:**

Rumen fluid, clarified .....	20.0mL
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**Preparation of Solution G:** Gas rumen fluid under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution H:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution H:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution I:****Composition per 10.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.5g
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**Preparation of Solution I:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 850.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 1000.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, 6.6mL of sterile solution F, 20.0mL of sterile solution G, and 10.0mL of sterile solution H. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution I. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfovibrio baarsii*.

### **Desulfovibrio Brackish Medium (DSMZ Medium 410)**

**Composition per liter:**

Solution A .....	980.0mL
Solution B .....	10.0mL
Solution C .....	10.0mL

**Solution A:****Composition per 980.0mL:**

NaCl .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
DL-Na-lactate .....	2.0g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg

pH 7.8 ± 0.2 at 25°C

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly.

**Solution B:****Composition per 10.0mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
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**Preparation of Solution B:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Solution C:****Composition per 10.0mL:**

Na-thioglycolate .....	0.1g
Ascorbic acid .....	0.1g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Bring solution A to a boil for a few minutes. Cool to room temperature while gassing with oxygen-free  $N_2$  gas. Add solutions B and C. Adjust pH to 7.8 with NaOH. Immediately distribute under  $N_2$  into anaerobic tubes. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Desulfovibrio giganteus* (*Desulfobacter giganteus*).

### *Desulfovibrio carbinolicus* Medium

**Composition** per 1001.0mL:

Solution A	870.0mL
Solution C	100.0mL
Solution D	10.0mL
Solution E (Vitamin solution)	10.0mL
Solution F	10.0mL
Solution B (Trace elements solution SL-10)	1.0mL
pH 7.1–7.4 at 25°C	

#### **Solution A:**

**Composition** per 870.0mL:

$Na_2SO_4$	3.0g
NaCl	1.0g
KCl	0.5g
$MgCl_2 \cdot 6H_2O$	0.4g
$NH_4Cl$	0.3g
$KH_2PO_4$	0.2g
$CaCl_2 \cdot 2H_2O$	0.15g
Yeast extract	0.1g
Casamino acids	0.1g
Resazurin	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80%  $N_2$  + 20%  $CO_2$ . Continue gassing until pH reaches below 6.0. Seal the flask under 80%  $N_2$  + 20%  $CO_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

$FeCl_2 \cdot 4H_2O$	1.5g
$CoCl_2 \cdot 6H_2O$	190.0mg
$MnCl_2 \cdot 4H_2O$	100.0mg
$ZnCl_2$	70.0mg
$Na_2MoO_4 \cdot 2H_2O$	36.0mg
$NiCl_2 \cdot 6H_2O$	24.0mg
$H_3BO_3$	6.0mg
$CuCl_2 \cdot 2H_2O$	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add  $FeCl_2 \cdot 4H_2O$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100%  $N_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

**Composition** per 100.0mL:

$NaHCO_3$	5.0g
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**Preparation of Solution C:** Add  $NaHCO_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80%  $N_2$  + 20%  $CO_2$ .

#### **Solution D:**

**Composition** per 10.0mL:

Sodium propionate	0.7g
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**Preparation of Solution D:** Add sodium propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $N_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E (Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100%  $N_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F:**

**Composition** per 10.0mL:

$Na_2S \cdot 9H_2O$	0.4g
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**Preparation of Solution F:** Add  $Na_2S \cdot 9H_2O$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100%  $N_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine 870.0mL of sterile solution A with 1.0mL of sterile solution B, 100.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, and 10.0mL of sterile solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 100%  $N_2$ .

**Use:** For the cultivation and maintenance of *Desulfovibrio carbinolicus*.

### *Desulfovibrio* Choline Medium (DSMZ Medium 272)

**Composition** per liter:

Solution A	980.0mL
Solution B	10.0mL
Solution C	10.0mL

#### **Solution A:**

**Composition** per 980.0mL:

Choline hydrochloride	5.0g
$MgSO_4 \cdot 7H_2O$	2.0g
Yeast extract	1.0g
$NH_4Cl$	1.0g
$Na_2SO_4$	1.0g
$K_2HPO_4$	0.5g
$CaCl_2 \cdot 2H_2O$	0.1g
Resazurin	1.0mg

pH 7.8 ± 0.2 at 25°C

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly.

#### **Solution B:**

**Composition** per 10.0mL:

$FeSO_4 \cdot 7H_2O$	0.5g
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**Preparation of Solution B:** Add  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

#### Solution C:

**Composition** per 10.0mL:

Na-thioglycolate .....	0.1g
Ascorbic acid .....	0.1g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Bring solution A to a boil for a few minutes. Cool to room temperature while gassing with oxygen-free  $\text{N}_2$  gas. Add solutions B and C. Adjust pH to 7.8 with NaOH. Immediately distribute under  $\text{N}_2$  into anaerobic tubes. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Desulfovibrio* spp.

#### *Desulfovibrio gabonensis* Medium

**Composition** per 1002.0mL:

NaCl .....	50.0g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ .....	3.3g
$\text{Na}_2\text{SO}_4$ .....	3.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	1.6g
KCl .....	0.3g
$\text{NH}_4\text{Cl}$ .....	0.3g
$\text{KH}_2\text{PO}_4$ .....	0.2g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.1g
Yeast extract .....	0.1g
Resazurin .....	0.5mg
Sodium lactate solution .....	10.0mL
$\text{NaHCO}_3$ solution .....	10.0mL
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution .....	10.0mL
Trace elements solution SL-10 with EDTA .....	1.0mL
Seven vitamin solution .....	1.0mL

pH 7.0–7.2 at 25°C

#### Sodium Lactate Solution:

**Composition** per 10.0mL:

Sodium lactate .....	2.5g
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**Preparation of Sodium Lactate Solution:** Add sodium lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### $\text{NaHCO}_3$ Solution:

**Composition** per 10.0mL:

$\text{NaHCO}_3$ .....	2.5g
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**Preparation of  $\text{NaHCO}_3$  Solution:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ Solution:

**Composition** per 10.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ .....	0.2g
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**Preparation of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10 with EDTA:

**Composition** per liter:

Disodium EDTA .....	3.0g
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ .....	1.5g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .....	190.0mg

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	100.0mg
$\text{ZnCl}_2$ .....	70.0mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	36.0mg
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ .....	24.0mg
$\text{H}_3\text{BO}_3$ .....	6.0mg
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ .....	2.0mg

#### Preparation of Trace Elements Solution SL-10 with EDTA:

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0.

#### Seven Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	0.3g
Thiamine-HCl .....	0.2g
Nicotinic acid .....	0.2g
Calcium DL-pantothenate .....	0.1g
Vitamin B <sub>12</sub> .....	0.1g
p-Aminobenzoic acid .....	80.0mg
Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100%  $\text{N}_2$ .

**Preparation of Medium:** Prepare and dispense medium under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Add components, except sodium lactate solution,  $\text{NaHCO}_3$  solution,  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, trace elements solution SL-10 with EDTA, and seven vitamin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Anaerobically distribute 9.7mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.1mL of sterile sodium lactate solution, 0.1mL of sterile  $\text{NaHCO}_3$  solution, 0.1mL of sterile  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, 0.01mL of sterile trace elements solution SL-10 with EDTA, and 0.01mL of sterile seven vitamin solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Desulfovibrio gabonensis*.

#### *Desulfovibrio giganteus* Medium

**Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Solution A:

**Composition** per 870.0mL:

NaCl .....	20.0g
$\text{Na}_2\text{SO}_4$ .....	3.0g
KCl .....	0.5g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.4g
$\text{NH}_4\text{Cl}$ .....	0.3g
$\text{KH}_2\text{PO}_4$ .....	0.2g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Continue gassing until pH reaches below 6.0. Seal the flask under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D:****Composition** per 10.0mL:

Sodium lactate .....	1.5g
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**Preparation of Solution D:** Add sodium acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, and solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfovibrio giganteus*.

***Desulfovibrio gigas* Medium****Composition** per 1001.0mL:

Solution A .....	950.0mL
Solution B .....	40.0mL
Solution C .....	6.0mL
Solution D (Vitamin solution) .....	5.0mL

pH 7.2 ± 0.2 at 25°C

**Solution A:****Composition** per 950.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	2.0g
Sodium (L)-lactate .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
H <sub>2</sub> SO <sub>4</sub> (1M solution) .....	1.0mL
Trace elements solution SL-6 .....	1.0mL

**Trace Elements Solution SL-6:****Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 40.0mL:

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution C:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Solution C:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	62.5g
Nicotinic acid .....	25.0mg
<i>p</i> -Aminobenzoic acid .....	12.5mg
Thiamine-HCl .....	12.5mg
Calcium DL-pantothenate .....	6.5mg
Biotin .....	2.5mg

**Preparation of Solution D (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine 950.0mL of sterile solution A with 40.0mL of sterile solution B, 6.0mL of sterile solution C, and 5.0mL of sterile solution D. Adjust pH to 7.2. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Desulfovibrio gigas*.

### *Desulfovibrio halophilus* Medium

**Composition** per 1154.0mL:

Solution A	1.0L
Solution H	67.0mL
Solution D	50.0mL
Solution I	13.0mL
Solution E	10.0mL
Solution G	10.0mL
Solution C (Selenite-tungstate solution)	2.0mL
Solution B (Trace elements solution SL-10)	1.0mL
Solution F	1.0mL

pH 6.8 ± 0.2 at 25°C

#### **Solution A:**

**Composition** per liter:

Na <sub>2</sub> SO <sub>4</sub>	4.0g
NH <sub>4</sub> Cl	0.25g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3-4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution C (Selenite-Tungstate Solution):**

**Composition** per liter:

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Solution C (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution D:**

**Composition** per 50.0mL:

NaHCO <sub>3</sub>	2.5g
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**Preparation of Solution D:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution E:**

**Composition** per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Filter sterilize.

#### **Solution F:**

**Composition** per 10.0mL:

Vitamin B <sub>12</sub>	0.5mg
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**Preparation of Solution F:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Filter sterilize.

#### **Solution G:**

**Composition** per 80.0mL:

Sodium-(L)-lactate	2.25g
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**Preparation of Solution G:** Add sodium-(L)-lactate to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. In a closed bottle, heat in a boiling water bath. Shake until stearic acid dissolves. Autoclave for 15 min at 15 psi pressure-121°C. On storage, solution will solidify and should be remelted before use.

#### **Solution H:**

**Composition** per liter:

NaCl	70.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	2.2g

**Preparation of Solution H:** Add components to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

#### **Solution I:**

**Composition** per 20.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.15g
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**Preparation of Solution I:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C.

**Preparation of Medium:** To 1.0L of sterile solution A, add in the following order: 1.0mL of sterile solution B, 2.0mL of sterile solution C, 50.0mL of sterile solution D, 10.0mL of sterile solution E, 1.0mL of sterile solution F, 10.0mL of sterile solution G, 67.0mL of sterile solution H, and 13.0mL of sterile solution I. Mix thoroughly. Final pH of medium should be 7.2. Prior to inoculation, add 10.0-20.0mg of sodium dithionite to 1.0L of medium.

**Use:** For the cultivation and maintenance of *Desulfovibrio halophilus*.



***Desulfovibrio halophilus* Medium****Composition** per liter:

NaCl .....	70.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaHCO <sub>3</sub> .....	2.5g
KCl .....	0.3g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Wolfe's vitamin solution .....	10.0mL
Sodium lactate .....	3.7mL
Trace elements solution SL-10 .....	1.0mL
pH 6.9–7.1 at 25°C	

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Wolfe's Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Add NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Anaerobically distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Desulfovibrio halophilus*.

***Desulfovibrio inopinatus* Medium  
(DSMZ Medium 799)****Composition** per 1008.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Yeast extract solution .....	5.0mL

Solution B (Trace elements solution SL-10) .....	1.0mL
Seven vitamin solution .....	1.0mL
Selenite-tungstate solution .....	1.0mL
pH 7.1–7.4 at 25°C	

**Solution A:****Composition** per 870.0mL:

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

**Solution D:****Composition** per 10.0mL:

Na-pyruvate .....	2.5g
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**Preparation of Solution D:** Add Na-pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.10mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume

to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

##### Composition per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Seven Vitamin Solution:

##### Composition per liter:

Pyridoxine hydrochloride ..... 300.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 200.0mg  
Nicotinic acid ..... 200.0mg  
Vitamin B<sub>12</sub> ..... 100.0mg  
Calcium pantothenate ..... 100.0mg  
*p*-Aminobenzoic acid ..... 80.0mg  
D(+)-Biotin ..... 20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

#### Selenite-Tungstate Solution:

##### Composition per liter:

NaOH ..... 0.5g  
Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O ..... 4.0mg  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Yeast Extract Solution:

##### Composition per 10.0mL:

Yeast extract ..... 1.0g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, 10.0mL solution F, 5.0mL yeast extract solution, 1.0mL selenite-tungstate solution, and 1.0mL seven vitamin solution. Distribute aseptically and anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into sterile tubes or bottles.

**Use:** For the cultivation of *Desulfovibrio inopinatus*.

### *Desulfovibrio magneticus* Medium (DSMZ Medium 896)

##### Composition per liter:

Na-fumarate ..... 0.58g  
Na-pyruvate ..... 0.44g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
NH<sub>4</sub>Cl ..... 0.06g  
Cysteine-HCl·H<sub>2</sub>O ..... 0.05g  
Vitamin solution ..... 8.0mL  
Trace elements solution ..... 4.0mL  
Fe(III)quinate solution ..... 2.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitrilotriacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O ..... 0.01g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Ferric Quinate Solution:

##### Composition per 100.0mL:

FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 0.45g  
Quinic acid ..... 0.19g

**Preparation of Ferric Quinate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except vitamin solution and ferric quinate solution to distilled/deionized water and bring volume to 990.0mLL. Purge medium with N<sub>2</sub> gas for 10 min. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 8.0mL vitamin solution and 2.0mL ferric quinate solution. Mix thoroughly. Adjust pH to 7.0. Purge medium with N<sub>2</sub> gas for 10 min. Under the same atmosphere, aseptically distribute medium to sterile tubes or bottles.

**Use:** For the cultivation of *Desulfovibrio magneticus*.

### *Desulfovibrio* Marine Medium (DSMZ Medium 163)

##### Composition per liter:

Solution A ..... 980.0mL  
Solution B ..... 10.0mL  
Solution C ..... 10.0mL

pH 7.8 ± 0.2 at 25°C

**Solution A:****Composition** per 980.0mL:

NaCl .....	25.0g
DL-Na-lactate .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to 980.0mL distilled/deionized water. Mix thoroughly.

**Solution B:****Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
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**Preparation of Solution B:** Add components to 10.0mL distilled/deionized water. Mix thoroughly.

**Solution C:****Composition** per 10.0mL:

Na-thioglycolate .....	0.1g
Ascorbic acid .....	0.1g

**Preparation of Solution C:** Add components to 10.0mL distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Bring solution A to a boil for a few minutes. Cool to room temperature while gassing with oxygen-free N<sub>2</sub> gas. Add solutions B and C. Mix thoroughly. Adjust pH to 7.8 with NaOH. Distribute under N<sub>2</sub> into anaerobic tubes. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Desulfovibrio vulgaris*, *Desulfovibrio desulfuricans*, *Desulfovibrio senezii*, and *Desulfovibrio vietnamensis*.

***Desulfovibrio Medium*****Composition** per 1056.5mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	5.3g
Sodium acetate .....	2.0g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> CO <sub>3</sub> solution .....	50.0mL
Solution 1 .....	10.0mL
Solution 2 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Solution 1:****Composition** per liter:

Nitrilotriacetic acid .....	12.8g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.17g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Solution 1:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with NaOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or NaOH. Add distilled/deionized water to 1.0L.

**Solution 2:****Composition** per 100.0mL:

Resazurin .....	0.2g
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**Preparation of Solution 2:** Add resazurin to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	8.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas with 100% N<sub>2</sub> for 20 min.

**HCl Solution:****Composition** per 100.0mL:

HCl .....	25.0mL
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**Preparation of HCl Solution:** Add HCl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Gas with 100% N<sub>2</sub> for 20 min.

**Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> Solution:****Composition** per 100.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	8.7g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Gas with 100% N<sub>2</sub> for 20 min.

**Preparation of Medium:** Add components—except Na<sub>2</sub>CO<sub>3</sub> solution, HCl solution, and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution—to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Anaerobically and aseptically add 50.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, 5.5mL of sterile HCl solution, and 1.0mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution. Mix thoroughly. Anaerobically and aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation, cultivation, and enrichment of *Desulfovibrio* species.

***Desulfovibrio Medium*****Composition** per liter of tap water:

Agar .....	15.0g
Glucose .....	5.0g
Peptone .....	5.0g
Beef extract .....	3.0g
MgSO <sub>4</sub> .....	1.5g
Na <sub>2</sub> SO <sub>4</sub> .....	1.5g
Yeast extract .....	0.2g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Sterilize by autoclaving for 15 min at 15 lbs pressure (121°C).

**Use:** For the cultivation and maintenance of *Desulfomaculum nigrificans*, *Desulfovibrio desulfuricans*, and *Desulfovibrio gigas*.

***Desulfovibrio Medium*****Composition** per liter:

Solution A .....	980.0mL
Solution B .....	10.0mL
Solution C .....	10.0mL

pH 7.8 ± 0.2 at 25°C

**Solution A:****Composition** per 980.0mL:

DL-Sodium lactate .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 7.4.

**Solution B:****Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
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**Preparation of Solution B:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Solution C:****Composition** per 10.0mL:

Ascorbic acid .....	0.1g
Sodium thioglycolate .....	0.1g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Combine 980.0mL of solution A, 10.0mL of solution B, and 10.0mL of solution C. Mix thoroughly. Adjust pH to 7.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Desulfovibrio desulfuricans*, *Desulfovibrio giganteus*, and *Desulfovibrio vulgaris*.

**Desulfovibrio Medium with Lactate****Composition** per liter:

Agar .....	15.0g
Lactate .....	10.0g
Glucose .....	5.0g
Peptone .....	5.0g
Beef extract .....	3.0g
MgSO <sub>4</sub> .....	1.5g
Na <sub>2</sub> SO <sub>4</sub> .....	1.5g
Yeast extract .....	0.2g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Desulfovibrio desulfuricans*.

**Desulfovibrio Medium with NaCl****Composition** per liter:

NaCl .....	30.0g
Agar .....	15.0g
Glucose .....	5.0g
Peptone .....	5.0g
Beef extract .....	3.0g
MgSO <sub>4</sub> .....	1.5g
Na <sub>2</sub> SO <sub>4</sub> .....	1.5g

Yeast extract .....	0.2g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Desulfovibrio desulfuricans* and *Desulfovibrio salexigens*.

**Desulfovibrio MG-1 Medium  
(DSMZ Medium 615)****Composition** per liter:

Na <sub>2</sub> SO <sub>4</sub> .....	4.5g
Glycerol .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
Na <sub>3</sub> -citrate·2H <sub>2</sub> O .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na-thioglycolate .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.06g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.04g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0mg
Resazurin .....	0.5mg

pH 6.9 ± 0.2 at 25°C

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas atmosphere. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Desulfovibrio* sp.

**Desulfovibrio sapovorans Medium****Composition** per 1009.0mL:

Solution A .....	850.0mL
Solution C .....	100.0mL
Solution G .....	20.0mL
Solution D .....	10.0mL
Solution E (Wolfe's vitamin solution) .....	10.0mL
Solution H .....	10.0mL
Solution F .....	6.6mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution I .....	0.4mL

pH 7.7 ± 0.2 at 25°C

**Solution A:****Composition** per 920.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction, and a pH of 6.0 is reached. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.10g
ZnCl <sub>2</sub> .....	0.070g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution C:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 90% N<sub>2</sub> + 10% CO<sub>2</sub> for 20 min.

**Solution D:****Composition** per 10.0mL:

Sodium butyrate .....	0.7g
Sodium caproate .....	0.3g
Sodium octanoate .....	0.15g

**Preparation of Solution D:** Prepare and dispense solution anaerobically under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution E (Wolfe's Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	0.01g
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Solution E (Wolfe's Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

**Solution F:****Composition** per 6.6mL:

AlCl <sub>3</sub> ·6H <sub>2</sub> O (4.9% solution) .....	5.0mL
Na <sub>2</sub> CO <sub>3</sub> (10.6% solution) .....	1.6mL

**Preparation of Solution F:** Combine both solutions. Mix thoroughly. Gas with 100% N<sub>2</sub>. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution G:****Composition** per 10.0mL:

Rumen fluid, clarified .....	20.0mL
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**Preparation of Solution G:** Gas rumen fluid under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution H:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution H:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution I:****Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.5g
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**Preparation of Solution I:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** Prepare and dispense medium under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. To 850.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 1000.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, 6.6mL of sterile solution F, 20.0mL of sterile solution G, and 10.0mL of sterile solution H. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution I. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfovibrio sapovorans*.

***Desulfovibrio sax Medium***  
**(DSMZ Medium 383a)**

**Composition** per 1022.6mL:

Solution A .....	930.0mL
Solution C .....	50.0mL
Solution E .....	20.0mL
Solution D .....	10.0mL
Solution G .....	10.0mL
Solution B .....	1.0mL
Solution F .....	1.0mL
Vitamin B <sub>12</sub> solution .....	0.5mL
Yeast extract solution .....	0.1mL
pH 7.3 at 25°C	

**Solution A:****Composition** per 930.0mL:

NaCl .....	21.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated, approximately 20 minutes. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution D:

**Composition per 10.0mL:**

Na-benzoate .....	0.5g
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**Preparation of Solution D:** Add Na-benzoate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize. Store anaerobically.

#### Solution E:

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Solution F:

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution F:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Solution G:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Vitamin B<sub>12</sub> Solution:

**Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	10.0mg
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**Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize.

#### Yeast Extract Solution:

**Composition per 10.0mL:**

Yeast extract .....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add solution B, solution C, solution D, solution E, Vitamin B<sub>12</sub> solution, yeast extract solution, solution F, and solution G to solution A in that order under N<sub>2</sub> gas. Adjust the pH to 7.3.

**Use:** For the cultivation of *Desulfotignum balticum* (*Desulfoarcus* sp.).

### Desulfovibrio SHV Medium

**Composition per 1003.0mL:**

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution G (Selenite-tungstate solution) .....	1.0mL
Solution H (Seven vitamin solution) .....	1.0mL
pH 7.1–7.4 at 25°C	

#### Solution A:

**Composition per 870.0mL:**

NaCl .....	7.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.3g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B (Trace Elements Solution SL-10):

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ .

#### Solution D:

**Composition** per 10.0mL:

Sodium lactate ..... 4.0g

**Preparation of Solution D:** Add sodium lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Calcium DL-pantothenate ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin  $\text{B}_{12}$  ..... 0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition** per 10.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  ..... 0.4g

**Preparation of Solution F:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution G (Selenite-Tungstate Solution):

**Composition** per liter:

$\text{NaOH}$  ..... 0.5g  
 $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  ..... 4.0mg  
 $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$  ..... 3.0mg

**Preparation of Solution G (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution H (Seven Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 0.3g  
Thiamine-HCl ..... 0.2g  
Nicotinic acid ..... 0.2g  
Calcium DL-pantothenate ..... 0.1g  
Vitamin  $\text{B}_{12}$  ..... 0.1g  
*p*-Aminobenzoic acid ..... 80.0mg  
Biotin ..... 20.0mg

**Preparation of Solution H (Seven Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, solution E, solution F, solution G, and solution H, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ .

**Use:** For the cultivation of *Desulfovibrio* species.

### *Desulfovibrio* sp. Medium (DSMZ Medium 200)

**Composition** per 1001.0mL:

Solution A ..... 870.0mL  
Solution C ..... 100.0mL  
Solution D ..... 10.0mL  
Solution E (Vitamin solution) ..... 10.0mL  
Solution F ..... 10.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
pH 6.8–7.0 at 25°C

#### Solution A:

**Composition** per 870.0mL:

$\text{NaCl}$  ..... 20.0g  
 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 3.1g  
 $\text{Na}_2\text{SO}_4$  ..... 3.0g  
 $\text{KH}_2\text{PO}_4$  ..... 0.2g  
 $\text{NH}_4\text{Cl}$  ..... 0.3g  
 $\text{KCl}$  ..... 0.5g  
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 0.15g  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 1.5g  
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 190.0mg  
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 100.0mg  
 $\text{ZnCl}_2$  ..... 70.0mg  
 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 36.0mg  
 $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 24.0mg  
 $\text{H}_3\text{BO}_3$  ..... 6.0mg  
 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

$\text{NaHCO}_3$  ..... 5.0g

**Preparation of Solution C:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  to remove dissolved oxygen.

#### Solution D:

**Composition** per 10.0mL:

*Na*-butyrate ..... 0.7g  
*Na*-caproate ..... 0.3g  
*Na*-octanoate ..... 0.15g

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl- $2\text{H}_2\text{O}$  ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*D*-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg

Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.10mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, and 10.0mL solution F. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels.

**Use:** For the cultivation of *Desulfovibrio* sp.

### *Desulfovibrio sulfodismutans* Medium (DSMZ Medium 386)

**Composition per 1002mL:**

Solution A .....	920.0mL
Solution C .....	50.0mL
Solution D .....	10.0mL
Solution F .....	10.0mL
Solution G .....	10.0mL
Solution B .....	1.0mL
Solution E .....	1.0mL

pH 7.1–7.4 at 25°C

#### Solution A:

**Composition per 920mL:**

NaCl .....	1.0g
KCl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
NH <sub>4</sub> Cl.....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution B:

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	7.7mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining

components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated, approximately 20 minutes. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution D:

**Composition per 10.0mL:**

Na-acetate·3H <sub>2</sub> O.....	0.3g
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**Preparation of Solution D:** Add Na<sub>2</sub>-acetate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize. Store anaerobically.

#### Solution E:

**Composition per 1mL:**

Ca-D-pantothenate .....	50.0µg
D(+)-Biotin .....	10.0µg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Solution F:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Solution G:

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution G:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add solution B, solution C, solution D, solution E, solution F, and solution G to solution A in that order under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas. Adjust the pH to 7.1–7.4. When growth has started feed culture again with same amount of solution G. After a further two days repeat feeding once more.

**Use:** For the cultivation of *Desulfovibrio sulfodismutans*.

### *Desulfovibrio zosteræ* Medium (DSMZ Medium 383c)

**Composition per 1023.5mL:**

Solution A .....	930.0mL
Solution C .....	50.0mL
Solution E .....	20.0mL
Solution D .....	10.0mL
Solution G .....	10.0mL
Solution B .....	1.0mL
Solution F .....	1.0mL
Selenite-tungstate solution .....	1.0mL
Vitamin B <sub>12</sub> solution .....	0.5mL

pH 7.3 at 25°C

#### Solution A:

**Composition per 930.0mL:**

NaCl .....	21.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g



MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	3.0g
KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution B:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	7.7mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated, approximately 20 min. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution D:

**Composition** per 10.0mL:

Na-lactate .....	1.25g
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**Preparation of Solution D:** Add Na<sub>2</sub>-lactate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize. Store anaerobically.

#### Solution E:

**Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid.....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
p-Aminobenzoic acid.....	80.0mg
D(+)-Biotin.....	20.0mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Solution F:

**Composition** per liter:

NaOH.....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Solution F:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Solution G:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Vitamin B<sub>12</sub> Solution:

**Composition** per 100.0mL:

Vitamin B <sub>12</sub> .....	10.0mg
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**Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize.

#### Selenite-Tungstate Solution

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add solution B, solution C, solution D, solution E, Vitamin B<sub>12</sub> solution, selenite-tungstate solution, solution F, and solution G to solution A in that order under N<sub>2</sub> gas. Adjust the pH to 7.3.

**Use:** For the cultivation of *Desulfovibrio zosteriae* (*Desulfovibrio* sp.).

### *Desulfovigra adipica* Medium (DSMZ Medium 868)

**Composition** per 2L:

Solution A .....	940.0mL
Solution E.....	50.0mL
Solution K .....	50.0mL
Solution G .....	20.0mL
Solution F .....	10.0mL
Solution M (Vitamin solution) .....	10.0mL
Solution H .....	7.0mL
Solution B.....	1.0mL
Solution C.....	1.0mL
Solution D .....	1.0mL
Solution I.....	1.0mL
Solution J (Trace elements solution SL-10) .....	1.0mL
Solution L (Selenite-tungstate solution).....	1.0mL
Solution N .....	variable

pH 7.1 ± 0.2 at 25°C

#### Solution A:

**Composition** per 940.0mL:

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution C:****Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Solution D:****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution E:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution F:****Composition** per 10mL:

Yeast extract .....	1.0g
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**Preparation of Solution F:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution G:****Composition** per 20mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.625g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution H:****Composition** per 10mL:

Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
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**Preparation of Solution H:** Add Na<sub>2</sub>SO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution I:****Composition** per 10mL:

Propanol .....	1.0g
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**Preparation of Solution I:** Add propanol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Filter sterilize.

**Solution J (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution J (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution K:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution K:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution L (Selenite-Tungstate Solution):****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution L (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution M (Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution M (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Solution N:****Composition** per 100mL:

Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
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**Preparation of Solution N:** Add  $\text{Na}_2\text{CO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$  gas mixture. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  gas atmosphere. Sequentially add 1.0mL solution B, 1.0mL solution C, 1.0mL solution D, 50.0mL solution E, 10.0mL solution F, 20.0mL solution G, 7.0mL solution H, 1.0mL solution I, 1.0mL solution J, 50.0mL solution K, 1.0mL solution L, and 10.0mL solution M, to 940.0mL solution A. Mix thoroughly. Adjust pH to 7.1 with solution N. Distribute anaerobically under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  into appropriate vessels.

**Use:** For the cultivation of *Desulfovibrio adipica* (*Desulfobacterium* sp.).

### *Desulfurella* Medium

#### Composition per liter:

Sulfur, powdered	10.0g
Sodium acetate	5.0g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.33g
KCl	0.33g
$\text{KH}_2\text{PO}_4$	0.33g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.33g
$\text{NH}_4\text{Cl}$	0.33g
Yeast extract	0.1g
Resazurin	1.0 mg
$\text{NaHCO}_3$ solution	40.0mL
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution	10.0mL
Wolfe's vitamin solution	10.0mL
Trace elements solution SL-10	1.0 mL
pH 6.8–7.0 at 25°C	

#### $\text{NaHCO}_3$ Solution:

##### Composition per 40.0mL:

$\text{NaHCO}_3$	2.0g
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**Preparation of  $\text{NaHCO}_3$  Solution:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Gas under 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ Solution:

##### Composition per 10.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	0.5g
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**Preparation of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

#### Wolfe's Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Calcium D-(+)-pantothenate	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Thioctic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Cyanocobalamin	100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution SL-10:

##### Composition per liter:

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	1.5g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	190.0mg

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	100.0mg
$\text{ZnCl}_2$	70.0mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	36.0mg
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	24.0mg
$\text{H}_3\text{BO}_3$	6.0mg
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	2.0mg
HCl (25% solution)	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Add components, except  $\text{NaHCO}_3$ , Wolfe's vitamin solution, and  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Adjust pH to 5.9. Do not autoclave. Sterilize medium by heating to 100°C for 1 hr on three consecutive days. Prior to inoculation, aseptically and anaerobically add 40.0mL of sterile  $\text{NaHCO}_3$  solution, 10.0mL of sterile Wolfe's vitamin solution, and 10.0mL of sterile  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution. Mix thoroughly. Final pH should be 6.8–7.0.

**Use:** For the cultivation of *Desulfurella* species, especially *Desulfurella acetivorans*.

### *Desulfurella* II Medium (DSMZ Medium 480c)

#### Composition per liter:

MOPS [3-(N-morpholino) propane sulfonic acid]	3.0g
Sulfur, powder	1.0g
$\text{NH}_4\text{Cl}$	0.33g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.33g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.33g
KCl	0.33g
$\text{KH}_2\text{PO}_4$	0.33g
Yeast extract	0.1g
Resazurin	1.0mg
$\text{NaHCO}_3$ solution	40.0mL
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution	10.0mL
Vitamin solution	10.0mL
Substrate solution	10.0mL
Trace elements solution SL-10	1.0mL
pH 6.9 ± 0.2 at 25°C	

#### $\text{NaHCO}_3$ Solution:

##### Composition per 10.0mL:

$\text{NaHCO}_3$	2.0g
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**Preparation of  $\text{NaHCO}_3$  Solution:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

#### Trace Elements Solution SL-10:

##### Composition per liter:

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	1.5g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	190.0mg
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	100.0mg
$\text{ZnCl}_2$	70.0mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	36.0mg
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	24.0mg
$\text{H}_3\text{BO}_3$	6.0mg
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	2.0mg
HCl (25% solution)	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly.

Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl-2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S-9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Substrate Solution:

##### Composition per 10.0mL:

Na-lactate	2.5g
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**Preparation of Substrate Solution:** Add Na-lactate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except sulfur, substrate solution, vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S-9H<sub>2</sub>O solution to 930.0mL distilled/deionized water. Mix thoroughly. Sparge for 30 min with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 5.9 with concentrated NaOH. Distribute under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into anaerobic tubes or bottles containing sulfur powder (100 mg S per 10.0mL medium). Autoclave 20 min at 110°C. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 10.0mL sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution, 10.0mL sterile vitamin solution, 10.0mL substrate solution, and 40.0mL sterile NaHCO<sub>3</sub> solution per liter medium.

**Use:** For the cultivation of *Thermoproteus uzoniensis* and *Desulfurella kamchatkensis*.

### *Desulfurella multipotens* Medium (DSMZ Medium 480a)

##### Composition per liter:

Sulfur, powder	10.0g
Na-butyrate	5.0g
NH <sub>4</sub> Cl	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.33g
KCl	0.33g
KH <sub>2</sub> PO <sub>4</sub>	0.33g
Yeast extract	0.1g
Resazurin	1.0mg
NaHCO <sub>3</sub> solution	40.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution	10.0mL

Vitamin solution	10.0mL
Trace elements solution SL-10	1.0mL
pH 6.9 ± 0.2 at 25°C	

#### NaHCO<sub>3</sub> Solution:

##### Composition per 10.0mL:

NaHCO <sub>3</sub>	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl-2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S-9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S-9H<sub>2</sub>O solution to 940.0mL distilled/deionized water. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 5.9 with concentrated NaOH. Sterilize medium by heating for 1 h at 90–100°C on three subsequent days. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Before use, aseptically and anaerobically add 10.0mL sterile vitamin solution, 10.0mL sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution, and 40.0mL sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfurella multipotens*.

***Desulfurococcus* Medium****Composition** per 1300.0mL:

Solution C .....	500.0mL
Solution B .....	450.0mL
Solution A .....	300.0mL
Solution D .....	50.0mL

**Solution A:****Composition** per 300.0mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.028g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 300.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Gas under 100% N<sub>2</sub> for 20 min.

**Solution B:****Composition** per 450.0mL:

Sulfur .....	5.0g
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**Preparation of Solution B:** Add sulfur to distilled/deionized water and bring volume to 450.0mL. Autoclave for 30 min at 0 psi pressure–100°C on three consecutive days. Gas under 100% N<sub>2</sub> for 20 min.

**Solution C:****Composition** per 500.0mL:

Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
Resazurin .....	1.0mg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Gas under 100% N<sub>2</sub> for 20 min.

**Solution D:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Gas under 100% N<sub>2</sub> for 20 min.

**Preparation of Medium:** Aseptically combine solutions A–D under nitrogen gas. Seal containers with butyl rubber stoppers.

**Use:** For the cultivation and maintenance of *Desulfurococcus mobilis* and *Desulfurococcus mucosus*.

***Desulfuromonas acetexigenes* Medium  
(DSMZ Medium 647)****Composition** per liter:

Sulfur, powdered .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
NaHCO <sub>3</sub> solution .....	33.0mL

Na-pyruvate solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Seven vitamin solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na-pyruvate Solution:****Composition** per 10.0mL:

Na-pyruvate .....	0.6g
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**Preparation of Na-pyruvate Solution:** Add Na-pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Seven Vitamin Solution:****Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D-(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Sulfur is sterilized by steaming for 3 hr on each of 3 successive days. Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except sulfur, NaHCO<sub>3</sub> solution, Na-pyruvate solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, seven vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 945.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Auto-

clave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 5.0g sterile sulfur, 33.0mL NaHCO<sub>3</sub> solution, 10.0mL Na-pyruvate solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 1.0mL seven vitamin solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Desulfuromonas thiophila* and *Desulfuromonas acetexigens*.

#### **Desulfuromonas acetoxidans Medium**

**Composition** per 1001.0mL:

Fumaric acid .....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
Sodium acetate .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	40.0mL
Trace elements solution SL-4 .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	6.0mL
Vitamin solution .....	5.0mL

pH 7.5 ± 0.2 at 25°C

#### **NaHCO<sub>3</sub> Solution:**

**Composition** per 40.0mL:

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Elements Solution SL-4:**

**Composition** per liter:

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

#### **Trace Elements Solution SL-6:**

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 6.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	62.5g
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Nicotinic acid .....	25.0mg
p-Aminobenzoic acid .....	12.5mg
Thiamine-HCl .....	12.5mg
Calcium DL-pantothenate .....	6.5mg
Biotin .....	2.5mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Gas under 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and vitamin solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 40.0mL of sterile NaHCO<sub>3</sub> solution, 6.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 5.0mL of sterile vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles or tubes. Fill completely, leaving only a small gas bubble.

**Use:** For the cultivation and maintenance of *Desulfuromonas acetoxidans*.

#### **Desulfuromonas Medium**

**Composition** per 1051.0mL:

Elemental sulfur slurry .....	10.0g
Solution 1 .....	1.0L
Solution 3 .....	40.0mL
Solution 4 .....	6.0mL
Solution 5 .....	5.0mL
Solution 2 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### **Solution 1:**

**Composition** per liter:

NaCl .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
HCl (2N solution) .....	4.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution 2:**

**Composition** per liter:

Disodium EDTA .....	5.2g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.9g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0g
ZnCl <sub>2</sub> .....	0.7g
H <sub>3</sub> BO <sub>3</sub> .....	0.62g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.36g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.24g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.17g

pH 6.5 ± 0.2 at 25°C

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **Solution 3:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of Solution 3:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thor-

oughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution 4:

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 5.0g

**Preparation of Solution 4:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution 5:

**Composition** per 200.0mL:

Pyridoxamine-HCl ..... 0.01g  
Nicotinic acid ..... 4.0mg  
*p*-Aminobenzoic acid ..... 2.0mg  
Thiamine ..... 2.0mg  
Cyanocobalamin ..... 1.0mg  
Pantothenic acid ..... 1.0mg  
Biotin ..... 0.5mg

**Preparation of Solution 5:** Add components to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

#### Elemental Sulfur Slurry:

**Composition** per 10.0g:

Sulfur flowers ..... 10.0g

**Preparation of Elemental Sulfur Slurry:** Add highly purified sulfur flowers to a mortar and grind to a fine powder. Add sufficient distilled/deionized water to produce a slurry. Distribute into 100.0mL screw-capped bottles in 20.0mL volumes. Autoclave for 30 min at 10 psi pressure–115°C. Decant supernatant solution. Reserve sulfur slurry.

**Preparation of Medium:** To 1.0L of cooled, sterile solution 1, aseptically add 1.0mL of sterile solution 2, 40.0mL of sterile solution 3, 6.0mL of sterile solution 4, and 5.0mL of sterile solution 5. Mix thoroughly. Adjust pH to 7.2. Aseptically distribute into sterile 50.0mL screw-capped bottles. Fill bottles completely with medium except for a pea-sized air bubble. Aseptically add a pea-sized piece of sulfur slurry to each 50.0mL of medium.

**Use:** For the isolation and cultivation of marine *Desulfuromonas* species.

### *Desulfuromonas Medium*

**Composition** per 1031.0mL:

Elemental sulfur slurry ..... 10.0g  
Solution 1 ..... 1.0L  
Solution 3 ..... 20.0mL  
Solution 4 ..... 6.0mL  
Solution 5 ..... 5.0mL  
Solution 2 ..... 1.0mL

pH 7.2 ± 0.2 at 25°C

#### Solution 1:

**Composition** per liter:

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.4g  
NH<sub>4</sub>Cl ..... 0.3g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
HCl (2*N* solution) ..... 4.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution 2:

**Composition** per liter:

Disodium EDTA ..... 5.2g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 1.9g  
FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.0g  
ZnCl<sub>2</sub> ..... 0.7g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.62g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.36g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.24g  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.17g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution 3:

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 10.0g

**Preparation of Solution 3:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution 4:

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 5.0g

**Preparation of Solution 4:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution 5:

**Composition** per 200.0mL:

Pyridoxamine-HCl ..... 0.01g  
Nicotinic acid ..... 4.0mg  
*p*-Aminobenzoic acid ..... 2.0mg  
Thiamine ..... 2.0mg  
Cyanocobalamin ..... 1.0mg  
Pantothenic acid ..... 1.0mg  
Biotin ..... 0.5mg

**Preparation of Solution 5:** Add components to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

#### Elemental Sulfur Slurry:

**Composition** per 10.0g:

Sulfur flowers ..... 10.0g

**Preparation of Elemental Sulfur Slurry:** Add highly purified sulfur flowers to a mortar and grind to a fine powder. Add sufficient distilled/deionized water to produce a slurry. Distribute into 100.0mL screw-capped bottles in 20.0mL volumes. Autoclave for 30 min at 10 psi pressure–115°C. Decant supernatant solution. Reserve sulfur slurry.

**Preparation of Medium:** To 1.0L of cooled, sterile solution 1, aseptically add 1.0mL of sterile solution 2, 40.0mL of sterile solution 3, 6.0mL of sterile solution 4, and 5.0mL of sterile solution 5. Mix thoroughly. Adjust pH to 7.2. Aseptically distribute into sterile 50.0mL screw-capped bottles. Fill bottles completely with medium except for a pea-sized air bubble. Aseptically add a pea-sized piece of sulfur slurry to each 50.0mL of medium.

**Use:** For the isolation and cultivation of freshwater *Desulfuromonas* species.

**Desulfofuomonas succinoxidans Medium****Composition per liter:**

NaCl	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.0g
KH <sub>2</sub> PO <sub>4</sub>	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.0g
NH <sub>4</sub> Cl	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
Resazurin	0.5mg
NaHCO <sub>3</sub> solution	50.0mL
Disodium fumarate solution	10.0mL
Sodium acetate solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Trace elements solution SL-10	1.0mL
Seven vitamin solution	1.0mL

pH 7.2 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 50.0mL:**

NaHCO <sub>3</sub>	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Disodium Fumarate Solution:****Composition per 10.0mL:**

Disodium fumarate	1.6g
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**Preparation of Disodium Fumarate Solution:** Add disodium fumarate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Sodium Acetate Solution:****Composition per 10.0mL:**

Sodium acetate	0.8g
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**Preparation of Sodium Acetate Solution:** Add sodium acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Seven Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl	0.3g
Thiamine-HCl	0.2g

Nicotinic acid	0.2g
Calcium DL-pantothenate	0.1g
Vitamin B <sub>12</sub>	0.1g
p-Aminobenzoic acid	80.0mg
Biotin	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, disodium fumarate solution, sodium acetate solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and seven vitamin solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile disodium fumarate solution, 10.0mL of sterile sodium acetate solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL of sterile seven vitamin solution. Mix thoroughly.

**Use:** For the cultivation of *Desulfofuomonas succinoxidans*.

**Desullobacterium Medium  
(DSMZ Medium 383)****Composition per 1012mL:**

Solution A	930.0mL
Solution C	50.0mL
Solution D	10.0mL
Solution E	10.0mL
Solution G	10.0mL
Solution B	1.0mL
Solution F	1.0mL

pH 7.0 at 25°C

**Solution A:****Composition per 930.0mL:**

NaCl	21.0g
Na <sub>2</sub> SO <sub>4</sub>	3.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.0g
KCl	0.5g
NH <sub>4</sub> Cl	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	300.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	7.7mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.



**Solution C:****Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated, approximately 20 minutes. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

**Solution D:****Composition** per 10.0mL:

Na-benzoate ..... 0.5g

**Preparation of Solution D:** Add Na<sub>2</sub>-benzoate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize. Store anaerobically.

**Solution E:****Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
 Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Nicotinic acid ..... 5.0mg  
 D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution F:****Composition** per liter:

NaOH ..... 0.5g  
 Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg

**Preparation of Solution F:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution G:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add solutions B, C, D, E, F, and G to solution A in that order under N<sub>2</sub> gas. Adjust the pH to 7.0.

**Use:** For the cultivation of *Desulfotobacterium* spp.

***Dethiosulfovibrio II Medium***  
**(DSMZ Medium 906)**

**Composition** per liter:

NaCl ..... 20.0g  
 Yeast extract ..... 5.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.0g  
 Na<sub>3</sub>-citrate·5H<sub>2</sub>O ..... 3.0g  
 Peptone ..... 2.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
 Resazurin ..... 0.5mg  
 Calcium chloride solution ..... 10.0mL  
 Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL  
 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution ..... 10.0mL  
 Trace elements solution SL-10 ..... 1.0mL  
 pH 6.7–6.8 at 25°C

**Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:****Composition** per 10mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 2.5g

**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
 HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Calcium Chloride Solution:****Composition** per 10.0mL:

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.2g

**Preparation of Calcium Chloride Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except calcium chloride solution, trace elements solution SL-10, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, to distilled/deionized water and bring volume to 969.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 30 min. Distribute under 100% N<sub>2</sub> into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically per 1.0L of medium add 10.0mL calcium chloride solution, 1.0mL trace elements solution SL-10, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. Mix thoroughly. The final pH should be 6.7–6.8.

**Use:** For the cultivation of *Dethiosulfovibrio* spp.

***Dethiosulfovibrio peptidovorans Medium***  
**(DSMZ Medium 786)**

**Composition** per 1085mL:

NaCl ..... 30.0g  
 Trypticase ..... 5.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.0g  
 Yeast extract ..... 1.0g  
 NH<sub>4</sub>Cl ..... 1.0g  
 L-Cysteine ..... 0.5g  
 Na-acetate ..... 0.5g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 0.3g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.3g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g

KCl.....	0.1g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	50.0mL
Na-thiosulfate solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	15.0mL
Trace elements solution .....	10.0mL

pH 7.3 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 20mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na-thiosulfate Solution:****Composition per 20.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
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**Preparation of Na-thiosulfate Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na-thiosulfate solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 20.0mL Na-thiosulfate solution, 50.0mL NaHCO<sub>3</sub> solution, and 15.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 7.3. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Dethiosulfovibrio peptidovorans*.

**Dextran Agar****Composition per liter:**

Minimal mineral base solution .....	700.0mL
Agar solution .....	200.0mL
Dextran-deoxyglucose solution .....	100.0mL

pH 4.0 ± 0.2 at 25°C

**Minimal Mineral Base Solution:****Composition per 700.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
CaCl <sub>2</sub> .....	0.1g
MnSO <sub>4</sub> .....	0.1g
NaCl .....	0.1g
Yeast extract .....	0.1g

**Preparation of Minimal Mineral Base Solution:**

Add components to distilled/deionized water and bring volume to 700.0mL. Mix thoroughly. Gently heat and bring to boiling. Before autoclaving pH is 4.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Agar Solution:****Composition per 200.0mL:**

Agar .....	10.0g
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**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Dextran-Deoxyglucose Solution:****Composition per 100.0mL:**

Dextran .....	10.0g
2-Deoxy-D-glucose .....	0.5g

**Preparation of Dextran-Deoxyglucose Solution:**

Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 700.0mL of sterile minimal mineral base solution, 200.0mL of sterile agar solution, and 100.0mL of sterile dextran-deoxyglucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Lipomyces starkeyi*.

**Dextrose Broth****Composition per liter:**

Pancreatic digest of casein .....	10.0g
Glucose .....	5.0g
NaCl .....	5.0g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of microorganisms based on their ability to ferment glucose. If desired, a Durham tube may be added to the test tubes to determine gas production.

**Dextrose Soil Agar  
(DSA)****Composition per liter:**

Soil .....	150.0g
Agar .....	20.0g
Glucose .....	5.0g

**Preparation of Medium:** Add soil to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman #1 filter paper. Bring volume to filtrate to 1.0L with distilled/deionized water. Mix thoroughly. Add agar and glucose. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Chaetomium globosum*.

### Diazotrophic Medium (RBA)

**Composition** per 1008.0mL:

Solution A .....	903.0mL
Solution B .....	50.0mL
Solution C .....	50.0mL
Solution D .....	5.0mL

pH 7.3 ± 0.2 at 25°C

#### Solution A:

**Composition** per 903.0mL:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NaCl .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	5.0mg
NaVO <sub>3</sub> ·2H <sub>2</sub> O .....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5mg
Trace elements solution SL-6 .....	3.0mL

#### Trace Elements Solution SL-6:

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 903.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Pour into sterile Petri dishes or distribute into sterile tubes.

#### Solution B:

**Composition** per 50.0mL:

DL-Malate .....	2.0g
Disodium succinate .....	1.0g
Yeast extract .....	0.05g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 50.0mL:

D-Mannitol .....	2.0g
D-Glucose .....	2.0g
Sodium pyruvate .....	1.0g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust pH to 7.3. Filter sterilize.

#### Solution D:

**Composition** per liter:

Pyridoxine-HCl .....	62.5g
Nicotinic acid .....	25.0mg
p-Aminobenzoic acid .....	12.5mg
Thiamine-HCl .....	12.5mg
Calcium DL-pantothenate .....	6.5mg
Biotin .....	2.5mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Gas under 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** To 903.0mL of sterile solution A, aseptically add 50.0mL of sterile solution B, 50.0mL of sterile solution C, and 5.0mL of sterile solution D. Mix thoroughly. Final pH should be 7.3. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Arthrobacter* species, *Azomonas* species, *Azorhizophilus paspali*, and *Azotobacter* species.

### Dibenzothiophene Mineral Medium

**Composition** per liter:

Beef extract .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
NH <sub>4</sub> Cl .....	2.0g
Dibenzothiophene .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.028g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of bacteria that can metabolize dibenzothiophene.

### Dichloroacetic Acid Medium No. 1

**Composition** per liter:

Yeast extract .....	10.0g
Glucose .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> PO <sub>4</sub> .....	1.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
2,4-Dichloroacetic acid .....	0.75g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·5H <sub>2</sub> O .....	0.01g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodococcus* species.

### Dichloroacetic Acid Medium No. 2

**Composition** per liter:

Yeast extract .....	10.0g
Glucose .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> PO <sub>4</sub> .....	1.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g

Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·5H <sub>2</sub> O .....	0.01g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.002g
2,4-Dichloroacetic acid .....	10.0mg
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas* species.

#### Dichloromethane Medium for *Hyphomicrobium*

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	4.1g
KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
Dichloromethane (methylene chloride) .....	1.0mL
Trace elements solution .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Trace Elements Solution:**

**Composition** per liter:

Ca(NO <sub>3</sub> ) <sub>2</sub> .....	25.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
H <sub>3</sub> BO <sub>3</sub> .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.0g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.25g
NH <sub>4</sub> VO <sub>3</sub> .....	0.1g

**Preparation of Medium:** Filter sterilize dichloromethane. Add components, except dichloromethane, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile dichloromethane. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Hyphomicrobium* species

#### *Dichotomicrobium thermohalophilum* Agar

**Composition** per liter:

Agar .....	18.0g
Disodium DL-malate .....	1.0g
Yeast extract .....	1.0g
Artificial seawater, 3× .....	960.0mL
Hutner's basal salts solution .....	20.0mL
NaHCO <sub>3</sub> solution .....	20.0mL
pH 7.0–7.2 at 25°C	

**Artificial Seawater, 3×:**

**Composition** per liter:

NaCl .....	70.43g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	31.86g
Na <sub>2</sub> SO <sub>4</sub> .....	11.75g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	4.35g
NaHCO <sub>3</sub> .....	2.88g
KCl .....	1.99g
KBr .....	0.29g
H <sub>3</sub> BO <sub>3</sub> .....	0.08g

**Preparation of Artificial Seawater, 3×:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Hutner's Basal Salts Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44" .....	50.0mL

**"Metals 44":**

**Composition** per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 20.0mL:

NaHCO <sub>3</sub> .....	3.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Dichotomicrobium thermohalophilum*.

#### *Dictyoglomus* Medium

**Composition** per liter:

Soluble starch .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	4.2g
Polypeptone .....	2.0g
Yeast extract .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
L-Cysteine-HCl·H <sub>2</sub> O .....	1.0g
Na <sub>2</sub> CO <sub>3</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.38g
CaCl <sub>2</sub> .....	0.05g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.039g
Resazurin .....	2.0mg
Trace metals .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Trace Metals:**

**Composition** per liter:

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.29g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.28g

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.24g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.017g

**Preparation of Trace Metals:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 6.0 with KOH. Mix thoroughly.

#### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine·HCl .....	0.01g
Thiamine·HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Wolfe's vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C under 100% N<sub>2</sub>. Aseptically add sterile Wolfe's vitamin solution. Mix thoroughly. Adjust pH to 7.2 if necessary. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Dictyoglomus thermophilum*.

#### *Dictyostelium Medium*

**Composition** per liter:

Glucose .....	15.4g
Agar .....	15.0g
Peptone .....	14.3g
Yeast extract .....	7.15g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	1.28g
KH <sub>2</sub> PO <sub>4</sub> .....	0.49g
pH 6.7 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.7. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Dictyostelium discoideum* and *Fusarium acuminatum*.

#### Dilute Potato Medium

**Composition** per 1090.0mL:

Glucose .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.12g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.05g
Peptone .....	0.05g
Potato decoction .....	100.0mL
pH 6.8 ± 0.2 at 25°C	

#### Potato Decoction:

**Composition** per liter:

Potato .....	20.0g
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**Preparation of Potato Decoction:** Peel and dice potato. Add to 1.0L of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 30 min. Filter through

Whatman #1 filter paper. Bring volume of filtrate to 1.0L with distilled/deionized water.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1090.0mL. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhizobacter daucus*.

#### Dinoflagellate Medium

**Composition** per 1020.0mL:

Seawater solution .....	1.0L
Basal solution .....	20.0mL
pH 7.8 ± 0.2 at 25°C	

#### Seawater Solution:

**Composition** per 1100mL:

Seawater .....	1010.0mL
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**Preparation of Seawater Solution:** Add seawater to distilled/deionized water and bring volume to 1100.0mL. Mix thoroughly. Adjust pH to 7.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Basal Solution:

**Composition** per 100.0mL:

Buffer salts solution .....	25.0mL
Fe solution .....	25.0mL
Vitamin solution .....	25.0mL
Metal solution .....	25.0mL

**Preparation of Basal Solution:** Adjust final pH to 7.8.

#### Buffer Salts Solution:

**Composition** per 25.0mL:

Tris·HCl .....	500.0mg
NaNO <sub>3</sub> .....	350.0mg
Sodium glycerophosphate·6H <sub>2</sub> O .....	50.0mg

**Preparation of Buffer Salts Solution:** Add components to distilled/deionized water and bring volume to 25.0mL. Adjust pH to 7.8. Mix thoroughly.

#### Fe Solution:

**Composition** per 500.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	351.0mg
EDTA .....	330.0mg

**Preparation of Fe Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

#### Vitamin Solution:

**Composition** per 25.0mL:

Vitamin B <sub>12</sub> .....	10.0μg
Biotin .....	5.0μg
Thiamine .....	0.5mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly.

#### Metal Solution:

**Composition** per 25.0mL:

H <sub>3</sub> BO <sub>3</sub> .....	114.0mg
EDTA .....	100.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	16.4mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	4.9mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.2mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.48mg

**Preparation of Metal Solution:** Add components, in the order listed, to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Adjust pH to 7.5.

**Preparation of Basal Solution:** Combine 25.0mL buffer salts solution, 25.0mL Fe solution, 25.0mL vitamin solution, and 25.0mL metal solution. Adjust pH to 7.8. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Aseptically combine 20.0mL of sterile basal solution with 1.0L of sterile seawater solution. Mix thoroughly. Aseptically distribute into sterile, screw-capped tubes or flasks.

**Use:** For the cultivation of *Amphidinium carteri*.

#### DM Medium

**Composition per liter:**

Starch, soluble.....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of myxobacteria.

#### DNase Medium

**Composition per liter:**

Agar.....	15.0g
Pancreatic digest of casein.....	10.0g
Peptic digest of animal tissue.....	10.0g
L-Arabinose.....	10.0g
NaCl.....	5.0g
Deoxyribonucleic acid.....	2.0g
Methyl Green.....	0.09g
Phenol Red.....	0.05g
Antibiotic solution.....	10.0mL

pH 7.3 ± 0.2 at 25°C

**Antibiotic Solution:**

**Composition per 10.0mL:**

Cephalothin.....	0.01g
Ampicillin.....	5.0mg
Colistimethate.....	5.0mg
Amphotericin B.....	2.5mg

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except antibiotic solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile components. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Serratia marcescens*.

#### DPM Medium (DSMZ Medium 737)

**Composition per liter:**

Na-propionate.....	1.20g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g
Chelated iron solution.....	1.8mL
Trace elements solution.....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Chelated Iron Solution:**

**Composition per liter:**

Na <sub>2</sub> -EDTA.....	7.56g
FeSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.54g

**Preparation of Chelated Iron Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.860
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.08g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.025g
CoCl <sub>2</sub> .....	0.025g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Frankia* sp.

#### DSIC Medium, Modified (DSMZ Medium 747)

**Composition per 994.0mL:**

Solution A.....	910.0mL
Solution B.....	70.0mL
Solution C.....	14.0mL

pH 7.0–7.1 at 25°C

**Solution A:**

**Composition per 960.0mL:**

NaCl.....	125.0g
K <sub>2</sub> SO <sub>4</sub> .....	2.5g
Na-acetate.....	2.0g
Yeast extract.....	0.75g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
NH <sub>4</sub> Cl.....	0.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	0.1g
MOPS buffer.....	10.0mL
Vitamin B <sub>12</sub> solution.....	1.0mL
Trace elements solution SL-10.....	1.0mL

**Vitamin B<sub>12</sub> Solution:**

**Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	10.0mg
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**Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min.

**Trace Elements Solution SL-10:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L.

Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### MOPS Buffer:

**Composition per 10mL:**

MOPS [3-( <i>N</i> -morpholino) propane sulfonic acid]	2.1g
Na-acetate	0.3g
EDTA	0.1g

**Preparation of MOPS Buffer:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Adjust to pH 7.2. Filter sterilize.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Gently heat and bring to boiling while continuing to sparge with 100% N<sub>2</sub>. Distribute about 13mL aliquots in 15mL Hungate tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### Solution B:

**Composition per 70.0mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.2g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 100% N<sub>2</sub>. Gently heat and bring to boiling while continuing to sparge with 100% N<sub>2</sub>. Distribute into a screw-capped bottle. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### Solution C:

**Composition per 14.0mL:**

NaHCO <sub>3</sub>	1.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 14.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Inject 1.0 ml of solution B and 0.2 ml of solution C in each tube of solution A.

**Use:** For the cultivation of *Rhodovibrio sodomensis*=*Rhodospirillum sodomense*.

### Dubos Agar with Filter Paper

**Composition per liter:**

Agar	15.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
KCl	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
NaNO <sub>3</sub>	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. Lay sterile strips of Whatman #1 filter paper on the surface of the agar.

**Use:** For the cultivation and maintenance of *Cytophaga hutchinsonii*.

### Dubos Mineral Medium

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub>	1.0g
KCl	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g

NaNO <sub>3</sub>	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### Dubos Salts Agar

**Composition per liter:**

Agar	15.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
KCl	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
NaNO <sub>3</sub>	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g
Filter paper strips, sterile	variable

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except filter paper strips, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. Aseptically place sterile filter paper strips onto the surface of the solidified medium.

**Use:** For the cultivation and maintenance of *Alteromonas* species, *Cellvibrio mixtus*, *Cellvibrio* species, *Cytophaga aurantiaca*, *Cytophaga hutchinsonii*, *Pseudomonas* species, and *Sporocytophaga myxococcoides*.

### Dubos Salts Agar with 1% NaCl

**Composition per liter:**

Agar	15.0g
NaCl	10.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
KCl	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
NaNO <sub>3</sub>	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	10.0mg
Filter paper strips	1 strip per tube

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position. Aseptically add a strip of sterile filter paper to the surface of each slant.

**Use:** For the cultivation of *Cytophaga* species.

### Dubos Salts Agar with Yeast Extract

**Composition per liter:**

Agar	15.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
Yeast extract	0.5g
KCl	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
NaNO <sub>3</sub>	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g
Filter paper strips, sterile	variable

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except filter paper strips, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to

boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. Aseptically place sterile filter paper strips onto the surface of the solidified medium.

**Use:** For the cultivation and maintenance of *Cellulomonas* species and *Cellvibrio* species.

#### Dubos Salts Broth

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaNO <sub>3</sub> .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Filter paper strips, sterile .....	variable
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except filter paper strips, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing a filter paper strip (filter paper strip should protrude above the surface of the broth). Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Cytophaga aurantiaca* and *Pseudomonas* species.

#### Dubos Salts Broth with Yeast Extract

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Yeast extract .....	0.5g
KCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaNO <sub>3</sub> .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Cytophaga aurantiaca*.

#### Dubos Salts Broth with Yeast Extract

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Yeast extract .....	0.5g
KCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaNO <sub>3</sub> .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Filter paper strips, sterile .....	variable
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except filter paper strips, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing a filter paper strip (filter paper strip should protrude above the surface of the broth). Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Cellulomonas* species and *Cellvibrio* species.

#### Dung Extract Agar (DSM Medium 781)

**Composition** per liter:

Agar .....	15.0g
Malt extract .....	5.0g
(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.72g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
Peptone .....	0.1g
Dung extract .....	100.0mL
pH 6.9 ± 0.2 at 25°C	

#### Dung Extract:

**Composition** per 150.0mL:

Horse dung .....	variable
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**Preparation of Dung Extract:** Add an average sized piece of horse dung to 150.0mL water. Gently heat and bring to boiling. Boil for 2h in a water bath. Filter. Use immediately.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Panaeolus cyaneus*.

#### E Agar (m-E Agar)

**Composition** per liter:

Yeast extract .....	30.0g
Agar .....	15.0g
NaCl .....	15.0g
Pancreatic digest of gelatin .....	10.0g
Esculin .....	1.0g
Nalidixic acid .....	0.25g
NaN <sub>3</sub> .....	0.15g
Cycloheximide .....	0.05g
TTC solution .....	15.0mL
pH 7.1 ± 0.2 at 25°C	

#### TTC Solution:

**Composition** per 15.0mL:

2,3,5-Triphenyltetrazolium chloride .....	0.15g
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**Preparation of TTC Solution:** Add triphenyltetrazolium chloride to distilled/deionized water and bring volume to 15.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except TTC solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile TTC solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Use:** For the isolation, cultivation, and enumeration of enterococci in water by the membrane filter method. It is used in conjunction with esculin iron agar.

#### E Medium for Anaerobes with 0.1% Cellobiose

**Composition** per 100.0mL:

Cellobiose .....	0.1g
Glucose .....	0.05g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.05g
Maltose .....	0.05g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.05g
Peptone .....	0.05g
Soluble starch .....	0.05g
Yeast extract .....	0.05g
Salts solution .....	50.0mL



Rumen fluid .....	30.0mL
Resazurin solution.....	0.4mL
pH 7.0 ± 0.2 at 25°C	

**Salts Solution:****Composition per liter:**

NaHCO <sub>3</sub> .....	10.0g
NaCl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> , anhydrous .....	0.2g
MgSO <sub>4</sub> .....	0.2g

**Preparation of Salts Solution:** Add CaCl<sub>2</sub> and MgSO<sub>4</sub> to approximately 300.0mL of distilled/deionized water. Mix thoroughly. Bring volume to 800.0mL with distilled/deionized water. Add remaining components. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Store at 4°C.

**Rumen Fluid:****Composition per 100.0mL:**

Rumen fluid .....	100.0mL
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**Preparation of Rumen Fluid:** Obtain the rumen contents from a cow that has been fed an alfalfa-hay ration. Filter rumen contents through two layers of cheesecloth. Store under 100% CO<sub>2</sub> in the refrigerator. The particulate material will settle out. Use only the supernatant liquid.

**Resazurin Solution:****Composition per 44.0mL:**

Resazurin .....	0.011g
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 44.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O, to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cool in an ice-water bath under 100% CO<sub>2</sub>. Add the L-cysteine-HCl-H<sub>2</sub>O. Adjust pH to 7.0 with 8N NaOH or 5N HCl. Anaerobically distribute into tubes under O<sub>2</sub>-free 100% N<sub>2</sub>. Cap tubes with butyl rubber stoppers. Place tubes in a press. Autoclave for 12 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation and maintenance of *Eubacterium cellulosolvens* and *Fibrobacter invrydinslid*.

### **E Medium for Anaerobes with Filtered Rumen Fluid and 0.1% Cellobiose**

**Composition per 100.0mL:**

Cellobiose .....	0.1g
Glucose .....	0.05g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.05g
Maltose .....	0.05g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.05g
Peptone .....	0.05g
Soluble starch .....	0.05g
Yeast extract .....	0.05g
Salts solution .....	50.0mL
Rumen fluid, filtered .....	30.0mL
Resazurin solution .....	0.4mL
pH 7.0 ± 0.2 at 25°C	

**Salts Solution:****Composition per liter:**

NaHCO <sub>3</sub> .....	10.0g
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NaCl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> (anhydrous) .....	0.2g
MgSO <sub>4</sub> .....	0.2g

**Preparation of Salts Solution:** Add CaCl<sub>2</sub> and MgSO<sub>4</sub> to approximately 300.0mL of distilled/deionized water. Mix thoroughly. Bring volume to 800.0mL with distilled/deionized water. Add remaining components. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Store at 4°C.

**Rumen Fluid:****Composition per 100.0mL:**

Rumen fluid .....	100.0mL
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**Preparation of Rumen Fluid:** Obtain the rumen contents from a cow that has been fed an alfalfa-hay ration. Filter rumen contents through two layers of cheesecloth. Store under 100% CO<sub>2</sub> in the refrigerator. The particulate material will settle out. Use only the supernatant liquid. Filter through a 0.20µm filter.

**Resazurin Solution:****Composition per 44.0mL:**

Resazurin .....	0.011g
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 44.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O, to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cool in an ice-water bath under 100% CO<sub>2</sub>. Add the L-cysteine-HCl-H<sub>2</sub>O. Adjust pH to 7.0 with 8N NaOH or 5N HCl. Anaerobically distribute into tubes under O<sub>2</sub>-free 100% N<sub>2</sub>. Cap tubes with butyl rubber stoppers. Place tubes in a press. Autoclave for 12 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation and maintenance of the *Fibrobacter* species.

### **E Medium for Anaerobes, Modified**

**Composition per 103.6mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	0.05g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.05g
Peptone .....	0.05g
Yeast extract .....	0.05g
Salts solution .....	50.0mL
Rumen fluid .....	30.0mL
Potassium phosphate buffer (1M, pH 6.5) .....	2.8mL
Hemin solution .....	1.0mL
Glucose-maltose solution .....	1.4mL
Starch solution .....	1.4mL
Resazurin (0.025% solution) .....	0.4mL
Vitamin K <sub>3</sub> solution .....	0.2mL
pH 6.5 ± 0.2 at 25°C	

**Salts Solution:****Composition per liter:**

NaHCO <sub>3</sub> .....	10.0g
NaCl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> , anhydrous .....	0.2g
MgSO <sub>4</sub> .....	0.2g

**Preparation of Salts Solution:** Add  $\text{CaCl}_2$  and  $\text{MgSO}_4$  to approximately 300.0mL of distilled/deionized water. Mix thoroughly. Bring volume to 800.0mL with distilled/deionized water. Add remaining components. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Store at 4°C.

#### Rumen Fluid:

**Composition** per 100.0mL:

Rumen fluid ..... 100.0mL

**Preparation of Rumen Fluid:** Obtain the rumen contents from a cow that has been fed an alfalfa-hay ration. Filter rumen contents through two layers of cheesecloth. Store under 100%  $\text{CO}_2$  in the refrigerator. The particulate material will settle out. Use only the supernatant liquid.

#### Hemin Solution:

**Composition** per 100.0mL:

Hemin ..... 0.05g  
NaOH (1N solution) ..... 1.0mL

**Preparation of Hemin Solution:** Add hemin to 1.0mL of 1N NaOH solution. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Glucose-Maltose Solution:

**Composition** per 10.0mL:

Glucose ..... 0.5g  
Maltose ..... 0.5g

**Preparation of Glucose-Maltose Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Starch Solution:

**Composition** per 10.0mL:

Starch, soluble ..... 0.5g

**Preparation of Starch Solution:** Add starch to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Resazurin Solution:

**Composition** per 44.0mL:

Resazurin ..... 0.011g

**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 44.0mL. Mix thoroughly.

#### Vitamin K<sub>3</sub> Solution:

**Composition** per 25.0mL:

Vitamin K<sub>3</sub> (menadione) ..... 0.0125g  
Ethanol, absolute ..... 25.0mL

**Preparation of Vitamin K<sub>1</sub> Solution:** Add Vitamin K<sub>3</sub> to 99.0mL of ethanol. Mix thoroughly.

**Preparation of Medium:** Filter sterilize potassium phosphate buffer. Add components—except L-cysteine-HCl-H<sub>2</sub>O, Vitamin K<sub>3</sub> solution, potassium phosphate buffer, glucose-maltose solution, and starch solution—to distilled/deionized water and bring volume to 98.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cool in an ice-water bath under  $\text{O}_2$ -free 97%  $\text{N}_2$  + 3%  $\text{H}_2$ . Add the L-cysteine-HCl-H<sub>2</sub>O and Vitamin K<sub>3</sub> solution. Mix thoroughly. Adjust pH to 6.5 with 8N NaOH or 5N HCl. Anaerobically distribute into tubes under  $\text{O}_2$ -free 97%  $\text{N}_2$  + 3%  $\text{H}_2$  in 7.0mL volumes. Cap tubes with butyl rubber stoppers.

Place tubes in a press. Autoclave for 12 min at 15 psi pressure–121°C with fast exhaust. Immediately prior to inoculation, aseptically add 0.2mL of filter-sterilized potassium phosphate buffer, 0.1mL of sterile glucose-maltose solution, and 0.1mL of sterile starch solution to each tube. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Bacteroides* species, *Butyrivibrio fibrisolvens*, *Clostridium methylpentosum*, *Eubacterium ruminantium*, *Lachnospira multipara*, *Micromonospora ruminantium*, *Prevotella ruminicola*, *Propionibacterium acidipropionici*, *Selenomonas* species, *Succinivibrio dextrinosolvens*, and *Treponema* species.

### E Medium for Anaerobes with 0.3% Phloroglucinol

**Composition** per 110.4mL:

$(\text{NH}_4)_2\text{SO}_4$  ..... 0.5g  
L-Cysteine-HCl-H<sub>2</sub>O ..... 0.05g  
Soluble starch ..... 0.05g  
Salts solution ..... 50.0mL  
Rumen fluid ..... 30.0mL  
Phloroglucinol solution ..... 30.0mL  
Resazurin solution ..... 0.4mL

pH 6.6 ± 0.2 at 25°C

#### Salts Solution:

**Composition** per liter:

$\text{NaHCO}_3$  ..... 10.0g  
NaCl ..... 2.0g  
 $\text{K}_2\text{HPO}_4$  ..... 1.0g  
 $\text{KH}_2\text{PO}_4$  ..... 1.0g  
 $\text{CaCl}_2$ , anhydrous ..... 0.2g  
 $\text{MgSO}_4$  ..... 0.2g

**Preparation of Salts Solution:** Add  $\text{CaCl}_2$  and  $\text{MgSO}_4$  to approximately 300.0mL of distilled/deionized water. Mix thoroughly. Bring volume to 800.0mL with distilled/deionized water. Add remaining components. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Store at 4°C.

#### Rumen Fluid:

**Composition** per 100.0mL:

Rumen fluid ..... 100.0mL

**Preparation of Rumen Fluid:** Obtain the rumen contents from a cow that has been fed an alfalfa-hay ration. Filter rumen contents through two layers of cheesecloth. Store under 100%  $\text{CO}_2$  in the refrigerator. The particulate material will settle out. Use only the supernatant liquid.

#### Phloroglucinol Solution:

**Composition** per 100.0mL:

Phloroglucinol ..... 1.0g

**Preparation of Phloroglucinol Solution:** Add phloroglucinol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Keep away from light.

#### Resazurin Solution:

**Composition** per 44.0mL:

Resazurin ..... 0.011g

**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 44.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O, to distilled/deionized water and bring

volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cool in an ice-water bath under 100% CO<sub>2</sub>. Add the L-cysteine·HCl·H<sub>2</sub>O. Adjust pH to 6.6 with 8N NaOH or 5N HCl. Anaerobically distribute into tubes under O<sub>2</sub>-free 100% N<sub>2</sub>. Cap tubes with butyl rubber stoppers. Place tubes in a press. Autoclave for 12 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation and maintenance of *Coprococcus* species.

### **E Medium for Anaerobes with 0.2% Rutin**

**Composition** per 110.4mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
L-Cysteine·HCl·H <sub>2</sub> O.....	0.05g
Soluble starch.....	0.05g
Salts solution.....	50.0mL
Rumen fluid.....	30.0mL
Rutin solution.....	30.0mL
Resazurin solution.....	0.4mL

pH 6.6 ± 0.2 at 25°C

#### **Salts Solution:**

**Composition** per liter:

NaHCO <sub>3</sub> .....	10.0g
NaCl.....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> , anhydrous.....	0.2g
MgSO <sub>4</sub> .....	0.2g

**Preparation of Salts Solution:** Add CaCl<sub>2</sub> and MgSO<sub>4</sub> to approximately 300.0mL of distilled/deionized water. Mix thoroughly. Bring volume to 800.0mL with distilled/deionized water. Add remaining components. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Store at 4°C.

#### **Rumen Fluid:**

**Composition** per 100.0mL:

Rumen fluid.....	100.0mL
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**Preparation of Rumen Fluid:** Obtain the rumen contents from a cow that has been fed an alfalfa-hay ration. Filter rumen contents through two layers of cheesecloth. Store under 100% CO<sub>2</sub> at 4°C. The particulate material will settle out. Use the liquid.

#### **Rutin Solution:**

**Composition** per 100.0mL:

Rutin.....	0.2g
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**Preparation of Rutin Solution:** Add rutin to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### **Resazurin Solution:**

**Composition** per 44.0mL:

Resazurin.....	0.011g
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 44.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except L-cysteine·HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Cool in an ice-water bath under 100%

CO<sub>2</sub>. Add the L-cysteine·HCl·H<sub>2</sub>O. Adjust pH to 6.6 with 8N NaOH or 5N HCl. Anaerobically distribute into tubes under O<sub>2</sub>-free 100% N<sub>2</sub>. Cap tubes with butyl rubber stoppers. Place tubes in a press. Autoclave for 12 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation of *Butyrivibrio* species.

### **EC Broth (*Escherichia coli* Broth) (EC Medium)**

**Composition** per liter:

Pancreatic digest of casein.....	20.0g
Lactose.....	5.0g
NaCl.....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
Bile salts mixture.....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into test tubes that contain an inverted Durham tube. Autoclave for 12 min at 15 psi pressure–121°C. Cool broth as quickly as possible.

**Use:** For the cultivation and differentiation of coliform bacteria at 37°C and of *Escherichia coli* at 45.5°C.

### **EC Broth with MUG**

**Composition** per liter:

Pancreatic digest of casein.....	20.0g
Lactose.....	5.0g
NaCl.....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
Bile salts mixture.....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g

4-Methylumbelliferyl-β-D-glucuronide (MUG).....	0.05g
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pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into test tubes that contain an inverted Durham tube in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the detection of *Escherichia coli* in water samples by a fluorogenic procedure.

### **EC Medium, Modified with Novobiocin**

**Composition** per liter:

Tryptone.....	20.0g
NaCl.....	5.0g
Lactose.....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
Bile salts.....	1.12g
Novobiocin supplement.....	10.0mL

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder and supplement from BD Diagnostic Systems.

**Novobiocin Supplement:****Composition** per 10.0mL:

Sodium novobiocin ..... 20.0mg

**Preparation of Novobiocin Supplement:** Add sodium novobiocin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except novobiocin supplement, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile novobiocin supplement. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Escherichia coli* O157:H7.

***Echinamoeba* Agar  
(ATCC Medium 2339)**

**Composition** per liter:

Agar ..... 18.0g  
 FeCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.552g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.49g  
 NaHCO<sub>3</sub> ..... 0.37g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.24g  
 Yeast extract ..... 0.2g  
 KCl ..... 8.6mg  
 KNO<sub>3</sub> ..... 0.16mg  
 Mineral solution ..... 10.0mL  
 Glycerol ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

**Mineral Solution:****Composition** per liter:

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.5g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.4g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.25g  
 (NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 LiCl ..... 0.3g  
 SnCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Echinamoeba* spp., e.g., *Echinamoeba therrmarum* n. sp., an extremely thermophilic amoeba thriving in hot springs.

***Echinamoeba* Agar**

**Composition** per liter:

Agar ..... 18.0g  
 FeCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.552g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.49g  
 NaHCO<sub>3</sub> ..... 0.37g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.24g  
 Yeast extract ..... 0.2g  
 KCl ..... 8.6mg  
 KNO<sub>3</sub> ..... 0.16mg  
 Mineral solution ..... 10.0mL  
 Glycerol ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

**Mineral Solution:****Composition** per liter:

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.5g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.4g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.25g  
 (NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 LiCl ..... 0.3g  
 SnCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with NaOH. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Echinamoeba* spp., e.g., *Echinamoeba therrmarum* n. sp., an extremely thermophilic amoeba thriving in hot springs.

***Echinamoeba* Broth**

**Composition** per liter:

FeCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.552g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.49g  
 NaHCO<sub>3</sub> ..... 0.37g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.24g  
 Yeast extract ..... 0.2g  
 KCl ..... 8.6mg  
 KNO<sub>3</sub> ..... 0.16mg  
 Mineral solution ..... 10.0mL  
 Glycerol ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

**Mineral Solution:****Composition** per liter:

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.5g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.4g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.25g  
 (NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 LiCl ..... 0.3g  
 SnCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Echinamoeba* spp., such as *Echinamoeba therrmarum*, an extremely thermophilic amoeba thriving in hot springs.

**ECM Agar**

**Composition** per liter:

Agar ..... 15.0g  
 NaCl ..... 6.0g  
*Escherichia coli* cells, washed ..... 1.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

***Ectothiorhodospira  
abdelmalekii* Medium**

**Composition** per 1010.0mL:

NaCl .....	120.0g
Na <sub>2</sub> SO <sub>4</sub> .....	15.0g
NaHCO <sub>3</sub> .....	10.0g
Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
Sodium acetate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.8g
NH <sub>4</sub> Cl .....	0.8g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SLA .....	1.0mL
Vitamin solution .....	1.0mL

pH 8.5 ± 0.2 at 25°C

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SLA:**

**Composition** per liter:

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	10.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution SLA:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2.0–3.0.

**Vitamin Solution VA:**

**Composition** per 100.0mL:

Nicotinic acid amide .....	35.0mg
Thiamine dichloride .....	30.0mg
<i>p</i> -Aminobenzoic acid .....	20.0mg
Biotin .....	10.0mg
Calcium DL-pantothenate .....	10.0mg
Pyridoxal-HCl .....	10.0mg
Vitamin B <sub>12</sub> .....	5.0mg

**Preparation of Vitamin Solution VA:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.5. Filter sterilize. Aseptically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Ectothiorhodospira abdelmalekii*.

***Ectothiorhodospira halochloris* Medium**

**Composition** per liter:

NaCl .....	180.0g
Na <sub>2</sub> SO <sub>4</sub> .....	20.0g
NaHCO <sub>3</sub> .....	14.0g
Na <sub>2</sub> CO <sub>3</sub> .....	6.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
Sodium succinate .....	1.0g
NH <sub>4</sub> Cl .....	0.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Yeast extract .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Vitamin solution VA .....	1.0mL
Trace elements solution SLA .....	1.0mL

pH 8.5–8.7 at 25°C

**Vitamin Solution VA:**

**Composition** per liter:

Nicotinamide .....	0.04g
Thiamine dichloride .....	0.03g
<i>p</i> -Aminobenzoic acid .....	0.02g
Biotin .....	0.01g
Calcium pantothenate .....	0.01g
Pyridoxal chloride .....	0.01g
Vitamin B <sub>12</sub> .....	5.0mg

**Preparation of Vitamin Solution VA:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SLA:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.07g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SLA:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.0 with 2N HCl.

**Preparation of Medium:** Add components, except trace elements solution SLA, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Filter sterilize. Aseptically add 1.0mL of sterile trace elements solution SLA. Mix thoroughly. Aseptically distribute into flasks or bottles. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the enrichment and isolation of *Ectothiorhodospira halochloris*.

***Ectothiorhodospira halophila* Medium**

**Composition** per liter:

NaCl .....	200.0g
NH <sub>4</sub> Cl .....	0.4g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
Na <sub>2</sub> CO <sub>3</sub> solution .....	100.0mL
Tris buffer (1M solution, pH 7.5) .....	30.0mL
Solution C .....	5.0mL
Potassium phosphate buffer (1M solution, pH 7.5) .....	3.0mL
Additional solution .....	2.5mL

pH 7.4–8.0 at 25°C

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 100.0mL:Na<sub>2</sub>CO<sub>3</sub> ..... 10.0g

**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution C:****Composition** per liter:MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0gCaCl<sub>2</sub>·2H<sub>2</sub>O ..... 3.3gFeCl<sub>3</sub>·4H<sub>2</sub>O ..... 1.1g(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O ..... 0.1g

Nitrilotriacetic acid ..... 10.0mg

Trace elements solution ..... 50.0mL

**Preparation of Solution C:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L.

**Trace Elements Solution:****Composition** per 100.0mL:ZnCl<sub>2</sub> ..... 0.52g

EDTA ..... 0.25g

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.08gFeCl<sub>3</sub>·4H<sub>2</sub>O ..... 0.03gCo(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.02gCuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.02gH<sub>3</sub>BO<sub>3</sub> ..... 0.01g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.0 with 2N HCl.

**Additional Solution:****Composition** per 50.0mL:NaS<sub>2</sub>O<sub>3</sub>·6H<sub>2</sub>O ..... 6.0g

Sodium succinate ..... 5.0g

Sodium ascorbate ..... 1.0g

**Preparation of Additional Solution:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution and additional solution, to distilled/deionized water and bring volume to 900.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically adjust pH to 7.4–7.8 with filter-sterilized HCl. Aseptically distribute into 50.0mL screw-capped bottles. Fill each bottle almost to the top, leaving a space of 2.8mL in the neck. Aseptically add 2.5mL of sterile additional solution to each bottle. Mix thoroughly.

**Use:** For the isolation and cultivation of *Ectothiorhodospira halophila*.

**Ectothiorhodospira Medium****Composition** per liter:

NaCl ..... 180.0g

Na<sub>2</sub>SO<sub>4</sub> ..... 20.0gNaHCO<sub>3</sub> ..... 14.0gNa<sub>2</sub>CO<sub>3</sub> ..... 6.0g

Sodium succinate ..... 1.0g

NH<sub>4</sub>Cl ..... 0.8gKH<sub>2</sub>PO<sub>4</sub> ..... 0.5gMgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1gCaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g

Feeding solution ..... 10.0mL

Trace elements solution SLA ..... 1.0mL

Vitamin solution VA ..... 1.0mL

pH 8.5–8.7 at 25°C

**Feeding Solution:****Composition** per 100.0mL:

NaCl ..... 10.0g

NaHCO<sub>3</sub> ..... 10.0gNa<sub>2</sub>S·9H<sub>2</sub>O ..... 5.0g

**Preparation of Feeding Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SLA:****Composition** per liter:FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.8gH<sub>3</sub>BO<sub>3</sub> ..... 0.5gCoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.25gZnCl<sub>2</sub> ..... 0.1gMnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.07gNaMoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.03gCuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.01gNa<sub>2</sub>SeO<sub>3</sub> ..... 0.01gNiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution SLA:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.0 with 2N HCl.

**Vitamin Solution VA:****Composition** per liter:

Nicotinamide ..... 0.04g

Thiamine dichloride ..... 0.03g

*p*-Aminobenzoic acid ..... 0.02g

Biotin ..... 0.01g

Calcium pantothenate ..... 0.01g

Pyridoxal chloride ..... 0.01g

Vitamin B<sub>12</sub> ..... 5.0mg

**Preparation of Vitamin Solution VA:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except trace elements solution SLA and feeding solution, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Filter sterilize. Aseptically add 1.0mL of sterile trace elements solution SLA. Mix thoroughly. Aseptically distribute into flasks or bottles. Completely fill bottles with medium except for a pea-sized air bubble. Prior to inoculation, aseptically remove a sufficient amount of medium to permit the addition of feeding medium. Add 1.0mL of feeding solution per each 100.0mL of medium.

**Use:** For the isolation and cultivation of *Ectothiorhodospira halochloris* and *Ectothiorhodospira halophila*.

**Ectothiorhodospira Medium****Composition** per liter:

NaCl ..... 130.0g

Na<sub>2</sub>SO<sub>4</sub> ..... 10.0g

Sodium acetate ..... 2.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.8g

Sodium carbonate buffer, (1M, pH 9.0) ..... 200.0mL

MgCl<sub>2</sub>·6H<sub>2</sub>O solution ..... 10.0mLNa<sub>2</sub>S·9H<sub>2</sub>O solution ..... 6.0mL

CaCl <sub>2</sub> ·2H <sub>2</sub> O solution.....	5.0mL
NH <sub>4</sub> Cl solution.....	4.0mL
SLA trace elements.....	1.0mL
VA vitamin solution.....	1.0mL
pH 9.0 ± 0.2 at 25°C	

**MgCl<sub>2</sub>·6H<sub>2</sub>O Solution:****Composition** per 10.0mL:

MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
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**Preparation of MgCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add 0.1g of MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Use freshly prepared solution.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:****Composition** per 10.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
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**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**NH<sub>4</sub>Cl Solution:****Composition** per 10.0mL:

NH <sub>4</sub> Cl.....	2.0g
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**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**SLA Trace Elements:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.25g
ZnCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.07g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.01g

**Preparation of SLA Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2–3.

**VA Vitamin Solution:****Composition** per 500.0mL:

Nicotinamide.....	0.175g
Thiamine·HCl.....	0.15g
<i>p</i> -Aminobenzoic acid.....	0.1g
Biotin.....	0.05g
Calcium pantothenate.....	0.05g
Pyridoxine·2HCl.....	0.05g
Cyanocobalamin.....	0.025g

**Preparation of VA Vitamin Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Preparation of Medium:** Add components—except MgCl<sub>2</sub>·6H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, CaCl<sub>2</sub>·2H<sub>2</sub>O solution, and NH<sub>4</sub>Cl solution—to distilled/deionized water

and bring volume to 975.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL of sterile MgCl<sub>2</sub>·6H<sub>2</sub>O solution, 6.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 5.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution, and 4.0mL of sterile NH<sub>4</sub>Cl solution. Mix thoroughly. Aseptically distribute into culture bottles. Incubate for 2 days before inoculation.

**Use:** For the cultivation and maintenance of *Ectothiorhodospira abdelmalekii* and *Ectothiorhodospira halochloris*.

**EIA Substrate****Composition** per liter:

Agar.....	15.0g
Esculin.....	1.0g
Ferric citrate.....	0.5g

pH 7.1 ± 0.1 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.1. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and enumeration of marine enterococci by the membrane filter method.

**Eijkman Lactose Medium****Composition** per liter:

Pancreatic digest of casein.....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
Lactose.....	3.0g
NaCl.....	2.5g

pH 6.8 ± 0.1 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into test tubes that contain an inverted Durham tube. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of *Escherichia coli* from other coliform organisms based on their ability to ferment lactose and produce gas.

**Ekho Lake Strains Medium  
(DSMZ Medium 621a)****Composition** per liter:

Agar.....	15.0g
Peptone.....	0.25g
Yeast extract.....	0.25g
Artificial seawater.....	250.0mL
Hutner's basal salts.....	20.0mL
Glucose solution.....	10.0mL
Vitamin solution.....	5.0mL

pH 7.3 ± 0.2 at 25°C

**Hutner's Basal Salts Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	29.7g
Nitilotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>7</sub> ·O <sub>24</sub> ·4H <sub>2</sub> O.....	9.25mg
"Metals 44".....	50.0mL

**“Metals 44”:****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of “Metals 44”:** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner’s Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Artificial Seawater:****Composition per liter:**

NaCl .....	23.477g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.981g
Na <sub>2</sub> SO <sub>4</sub> .....	3.917g
CaCl <sub>2</sub> .....	1.12g
KCl .....	664.0mg
NaHCO <sub>3</sub> .....	192.0mg
H <sub>3</sub> BO <sub>3</sub> .....	26.0mg
SrCl <sub>2</sub> .....	24.0mg
KBr .....	6.0mg
NaF .....	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	20.0mg
Riboflavin .....	10.0mg
Nicotinamide .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	10.0mg
Ca-pantothenate .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	10.0mg
Biotin .....	4.0mg
Folic acid .....	4.0mg
Vitamin B <sub>12</sub> .....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Store in the dark at 5°C.

**Glucose Solution:****Composition per 10mL:**

Glucose .....	0.25g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except artificial seawater, glucose solution, and vitamin solution to distilled/deionized water and bring volume to 735.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 60°C. Warm artificial seawater to 55°C. Aseptically add 250.0mL warm artificial seawater. Mix thoroughly. Adjust pH to 7.3. Aseptically add

10.0mL glucose solution and 5.0 mL vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Nocardioides aquaticus*, *Antarctobacter heliothermus*, *Sulfitobacter brevis*, *Roseovarius tolerans*, *Staleyia guttiformis*, *Roseovarius tolerans*, *Friedmanniella lacustris*, and *Nesterenkonia lacusekhoensis*.

**EMB Agar****(Eosin Methylene Blue Agar)****Composition per liter:**

Agar .....	13.5g
Pancreatic digest of casein .....	10.0g
Lactose .....	5.0g
Sucrose .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Eosin Y .....	0.4g
Methylene Blue .....	0.065g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the isolation, cultivation, and differentiation of Gram-negative enteric bacteria based on lactose fermentation. Bacteria that ferment lactose, especially the coliform bacterium *Escherichia coli*, appear as colonies with a green metallic sheen or blue-black to brown color. Bacteria that do not ferment lactose appear as colorless or transparent, light purple colonies.

**EMB Agar, Modified****(Eosin Methylene Blue Agar, Modified)****Composition per liter:**

Agar .....	15.0g
Lactose .....	10.0g
Pancreatic digest of gelatin .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Eosin Y .....	0.4g
Methylene Blue .....	0.065g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C. Shake medium to oxidize methylene blue. Pour into sterile Petri dishes. Swirl flask while pouring plates to distribute precipitate.

**Use:** For the isolation, cultivation, and differentiation of Gram-negative enteric bacteria based on lactose fermentation. Bacteria that ferment lactose, especially the coliform bacterium *Escherichia coli*, appear as colonies with a green metallic sheen or blue-black to brown color. Bacteria that do not ferment lactose appear as colorless or transparent, light purple colonies.



### Emerson Agar (ATCC Medium 199)

#### Composition per liter:

Agar .....	20.0g
Glucose .....	10.0g
Beef extract .....	4.0g
Pancreatic digest of gelatin .....	4.0g
NaCl .....	2.5g
Yeast extract .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 13 psi pressure–118°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and maintenance of members of the Actinomycetaceae, Streptomycetaceae, and molds. For the cultivation and maintenance of *Arthrobacter* species, *Microbispora rosea*, *Micromonospora coerulea*, *Mycobacterium* species, *Nocardia asteroides*, *Nocardiopsis dassonvillei*, *Pseudonocardia thermophila*, *Staphylococcus epidermidis*, *Streptomyces flaveus*, *Streptomyces olivaceus*, *Streptomyces thermoviolaceus*, *Streptomyces thermovulgaris*, and *Streptomyces vendargensis*.

### Endo Agar

#### Composition per liter:

Agar .....	15.0g
Lactose .....	10.0g
Peptic digest of animal tissue .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.5g
Na <sub>2</sub> SO <sub>3</sub> .....	2.5g
Basic Fuchsin .....	0.5g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Pour into sterile Petri dishes. Swirl flask while pouring plates to keep precipitate in suspension. Protect from the light.

**Use:** For the selective isolation, cultivation, and differentiation of coliform and other enteric microorganisms based on their ability to ferment lactose. Lactose-fermenting bacteria appear as dark red colonies with a gold metallic sheen. Lactose-nonfermenting bacteria appear as colorless or translucent colonies.

### Endo Agar

#### Composition per liter:

Agar .....	10.0g
Lactose .....	10.0g
Peptic digest of animal tissue .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.5g
Na <sub>2</sub> SO <sub>3</sub> .....	2.5g
Basic Fuchsin solution .....	4.0mL

pH 7.5 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

### Basic Fuchsin Solution:

#### Composition per 10.0mL:

Basic Fuchsin .....	1.0g
Ethanol (95% solution) .....	10.0mL

**Preparation of Basic Fuchsin Solution:** Add Basic Fuchsin to 10.0mL of ethanol. Mix thoroughly.

**Caution:** Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Pour into sterile Petri dishes. Swirl flask while pouring plates to keep precipitate in suspension. Protect from the light.

**Use:** For the selective isolation, cultivation, and differentiation of coliform and other enteric microorganisms based on their ability to ferment lactose. Lactose-fermenting bacteria appear as dark red colonies with a gold metallic sheen. Lactose-nonfermenting bacteria appear as colorless or translucent colonies.

### Endo Agar, LES

#### (Endo Agar, Laurance Experimental Station)

#### (m-Endo Agar, LES)

#### (m-LES, Endo Agar)

#### Composition per liter:

Agar .....	14.0g
Lactose .....	9.4g
Peptones (pancreatic digest of casein 65% and yeast extract 35%) .....	7.5g
NaCl .....	3.7g
Pancreatic digest of casein .....	3.7g
Peptic digest of animal tissue .....	3.7g
K <sub>2</sub> HPO <sub>4</sub> .....	3.3g
Na <sub>2</sub> SO <sub>3</sub> .....	1.6g
Yeast extract .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
Basic Fuchsin .....	0.8g
Sodium lauryl sulfate .....	0.05g
Ethanol .....	20.0mL

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add ethanol to approximately 900.0mL of distilled/deionized water. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile 60mm Petri dishes in 4.0mL volumes. Protect from the light.

**Use:** For the cultivation and enumeration of coliform bacteria by the membrane filter method.

### Endo Broth (m-Endo Broth)

#### Composition per liter:

Lactose .....	12.5g
Peptone .....	10.0g
NaCl .....	5.0g
Pancreatic digest of casein .....	5.0g
Peptic digest of animal tissue .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.375g
Na <sub>2</sub> SO <sub>3</sub> .....	2.1g
Yeast extract .....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.375g
Basic Fuchsin .....	1.05g
Sodium deoxycholate .....	0.1g
Ethanol (95% solution) .....	20.0mL

pH 7.2 ± 0.1 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add ethanol to approximately 900.0mL of distilled/deionized water. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Rapidly cool broth below 45°C. Do not autoclave. Use 1.8–2.0mL for each filter pad. Protect from the light. Prepare broth freshly.

**Use:** For the cultivation and enumeration of coliform bacteria from water by the membrane filter method.

### Enriched *Cytophaga* Agar

#### Composition per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	2.0g
Beef extract .....	0.5g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Cytophaga arvensicola*, *Cytophaga johnsonae*, *Cytophaga psychrophila*, *Cytophaga* species, *Cytophaga succinicans*, *Flavobacterium aquatile*, *Flavobacterium branchiophila*, *Flexibacter aurantiacus*, *Flexibacter canadensis*, *Flexibacter* species, *Pseudomonas echinoides*, *Psychrobacter immobilis*, *Xanthobacter autotrophicus*, and *Zoogloea ramigera*.

### Enrichment Broth for *Aeromonas hydrophila*

#### Composition per liter:

NaCl .....	5.0g
Maltose .....	3.5g
Yeast extract .....	3.0g
Bile salts No. 3 .....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.3g
Bromothymol Blue .....	0.03g
Novobiocin .....	5.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and enrichment of *Aeromonas hydrophila*.

### Entamoeba Medium (Endamoeba Medium)

#### Composition per liter:

Liver infusion .....	272.0g
Rice powder .....	14.2g
Agar .....	11.0g
Proteose peptone .....	5.5g
Sodium glycerophosphate .....	3.0g
NaCl .....	2.7g
Horse serum .....	50.0mL

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

### Rice Powder:

#### Composition per 15.0g:

Rice powder .....	15.0g
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**Preparation of Rice Powder:** Sterilize rice powder at 160°C for 60 min. Do not overheat or rice powder will scorch.

**Preparation of Medium:** Add components, except horse serum and rice powder, to distilled/deionized water and bring volume to 994.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 7.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position. Aseptically add enough sterile horse serum to each tube to cover about half the slant. Aseptically add 0.1g of sterile rice powder to each tube.

**Use:** For the cultivation of *Entamoeba histolytica*.

### Enteric Fermentation Base (Fermentation Base for *Campylobacter*)

#### Composition per liter:

Peptic digest of animal tissue .....	10.0g
NaCl .....	5.0g
Beef extract .....	3.0g
Carbohydrate solution .....	100.0mL
Andrade's indicator .....	10.0mL

pH 7.2 ± 0.1 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

### Carbohydrate Solution:

#### Composition per 100.0mL:

Carbohydrate .....	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Glucose, lactose, mannitol, sucrose, adonitol, arabinose, cellobiose, dulcitol, glycerol, inositol, salicin, xylose, or other carbohydrates may be used. For the preparation of expensive carbohydrate solutions (adonitol, arabinose, cellobiose, dulcitol, glycerol, inositol, salicin, or xylose), 5.0g of carbohydrate per 100.0mL of distilled/deionized water may be used.

**Andrade's Indicator:****Composition** per 100.0mL:

NaOH (1N solution).....	16.0mL
Acid Fuchsin .....	0.1g

**Caution:** Acid Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Andrade's Indicator:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes that contain an inverted Durham tube in 9.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 1.0mL of sterile carbohydrate solution per tube. Mix thoroughly.

**Use:** For the cultivation and differentiation of a variety of bacteria based on their ability to ferment different carbohydrates. Bacteria that produce acid from carbohydrate fermentation turn the medium dark pink to red. Bacteria that produce gas have a bubble trapped in the Durham tube.

**Enterobacter Medium****Composition** per 800.0mL:

Casein hydrolysate.....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.4g
K <sub>2</sub> SO <sub>4</sub> .....	1.0g
Yeast extract.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
MgSO <sub>4</sub> .....	0.5g
Glycerol .....	20.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Enterobacter* species and *Klebsiella pneumoniae*.

**Enterococci Confirmatory Agar****Composition** per liter:

Agar .....	15.0g
Glucose .....	5.0g
Pancreatic digest of casein.....	5.0g
Yeast extract.....	5.0g
NaN <sub>3</sub> .....	0.4g
Methylene Blue.....	10.0mg
Enterococci confirmatory broth .....	variable
pH 8.0 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position. Add sufficient amount of Enterococci confirmatory broth (see below) to cover half the slant.

**Use:** For the identification of enterococci from water by the confirmatory test.

**Enterococci Presumptive Broth****Composition** per liter:

Glucose.....	5.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
NaN <sub>3</sub> .....	0.4g
Bromothymol Blue.....	32.0mg
pH 8.4 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and identification of enterococci from water by the presumptive test. Bacteria that produce acid and turn the medium yellow and turbid after incubation at 45°C are presumptive enterococci.

**Enterococcus Agar  
(m-Enterococcus Agar)  
(Azide Agar)****Composition** per liter:

Pancreatic digest of casein .....	15.0g
Agar .....	10.0g
Papaic digest of soybean meal .....	5.0g
Yeast extract .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
Glucose.....	2.0g
NaN <sub>3</sub> .....	0.4g
Triphenyltetrazolium chloride.....	0.1g
pH 7.2 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Cool to 45°–50°C. Do not autoclave. Pour into sterile Petri dishes.

**Use:** For the isolation, cultivation, and enumeration of enterococci in water and sewage by the membrane filter method. For the direct plating of specimens for the detection and enumeration of fecal streptococci.

**Erwinia amylovora Selective Medium****Composition** per liter:

Agar.....	20.0g
Mannitol.....	10.0g
L-Asparagine .....	3.0g
Sodium taurocholate.....	2.5g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Nicotinic acid .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Nitritotriacetic acid.....	10.0mL
Actidione (cycloheximide) solution .....	10.0mL
Bromothymol Blue.....	9.0mL

Neutral Red .....	2.5mL
TiNO <sub>3</sub> solution .....	1.75mL
Tergitol 7 .....	0.1mL
pH 7.2–7.3 at 25°C	

#### Cycloheximide Solution:

##### Composition per 10.0mL:

Cycloheximide .....	0.05g
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**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### TiNO<sub>3</sub> Solution:

##### Composition per 10.0mL:

TiNO <sub>3</sub> .....	0.1g
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**Preparation of TiNO<sub>3</sub> Solution:** Add TiNO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except TiNO<sub>3</sub> solution and cycloheximide solution, to distilled/deionized water and bring volume to 988.25mL. Mix thoroughly. Adjust pH to 7.2–7.3. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 1.75mL of sterile TiNO<sub>3</sub> solution and 10.0mL of sterile cycloheximide solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Erwinia amylovora*.

#### *Erwinia* Fermentation Medium

##### Composition per liter:

Lactose .....	45.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.6g
KH <sub>2</sub> PO <sub>4</sub> .....	1.8g
Yeast extract .....	1.8g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.46g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.04g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.9mg
CoCl <sub>2</sub> .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with NaOH (approximately 0.18g). Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rahnella aquatilis*.

#### *Erwinia* Medium D3

##### Composition per liter:

Agar .....	15.0g
Arabinose .....	10.0g
Sucrose .....	10.0g
LiCl .....	7.0g
Casein hydrolysate .....	5.0g
NaCl .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
Acid Fuchsin .....	0.1g

Bromothymol Blue .....	0.06g
Sodium dodecyl sulfate .....	0.05g
pH 7.0 ± 0.2 at 25°C	

**Caution:** Acid Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 8.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. The pH after autoclaving should be 7.0. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Erwinia* species.

#### *Erwinia* Selective Medium

##### Composition per liter:

Agar .....	15.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Eosin Y .....	0.4g
Methylene Blue .....	0.065g
Glycerol .....	10.0mL
Antibiotic solution .....	10.0mL

#### Antibiotic Solution:

##### Composition per 10.0mL:

Cycloheximide .....	0.25g
Novobiocin .....	0.04g
Neomycin sulfate .....	0.04g

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except antibiotic solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of *Erwinia* species.

#### *Erwinia tracheiphila* Agar

##### Composition per liter:

Agar .....	15.0g
Glucose .....	10.0g
Peptone .....	10.0g
Beef extract .....	5.0g
Yeast extract .....	1.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Erwinia tracheiphila*.

#### *Erythrobacter longus* Medium

##### Composition per liter:

Peptone .....	2.0g
Proteose peptone No. 3 .....	1.0g
Papaic digest of soybean meal .....	1.0g
Yeast extract .....	1.0g

Artificial seawater .....	700.0mL
Ferric citrate solution .....	2.0mL
pH 7.5 ± 0.2 at 25°C	

**Ferric Citrate Solution:****Composition per 10.0mL:**

Ferric citrate .....	0.5g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Erythrobacter longus*.

***Erythromicrobium roseococcus* Medium  
(DSMZ Medium 767)**

**Composition per 1001mL:**

Na-acetate .....	1.0g
Yeast extract .....	1.0g
Peptone .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KCl .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements SL-6 .....	1.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL
pH 7.5-7.8 at 25°C	

**Vitamin B<sub>12</sub> Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	2.0mg
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**Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5-7.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10mL vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Erythrobacter litoralis*, *Erythromicrobium ramosum*, and *Roseococcus thiosulfatophilus*.

**Esculin Agar**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	13.0g

NaCl .....	5.0g
Yeast extract .....	5.0g
Heart muscle, solids from infusion .....	2.0g
Esculin .....	1.0g
Ferric citrate .....	0.5g
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into screw-capped tubes in 3.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Use:** For the cultivation and differentiation of bacteria based on their ability to hydrolyze esculin and produce H<sub>2</sub>S. Bacteria that hydrolyze esculin appear as colonies surrounded by a reddish-brown to dark brown zone. Bacteria that produce H<sub>2</sub>S appear as black colonies.

**Esculin Iron Agar**

**Composition per liter:**

Agar .....	15.0g
Esculin .....	1.0g
Ferric ammonium citrate .....	0.5g
pH 7.1 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and identification of enterococci based on their ability to hydrolyze esculin. Used in conjunction with E agar and the membrane filter method.

**Ethyl Violet Azide Broth  
(EVA Broth)**

**Composition per liter:**

Pancreatic digest of casein .....	13.5g
Yeast extract .....	6.5g
Glucose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.7g
KH <sub>2</sub> PO <sub>4</sub> .....	2.7g
NaN <sub>3</sub> .....	0.4g
Ethyl Violet .....	0.83mg
pH 7.0 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enumeration of enterococci from water and other specimens. Fecal enterococci turn the medium turbid with a purple sediment on the bottom of the tube.

### Ethyl Violet Azide Broth (EVA Broth)

#### Composition per liter:

Tryptose .....	20.0g
Glucose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.7g
KH <sub>2</sub> PO <sub>4</sub> .....	2.7g
NaN <sub>3</sub> .....	0.4g
Ethyl Violet .....	0.83mg

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enumeration of enterococci from water and other specimens. Fecal enterococci turn the medium turbid with a purple sediment on the bottom of the tube.

### Ethyl Violet Azide Broth (EVA Broth)

#### Composition per liter:

Tryptose .....	20.0g
Glucose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.7g
KH <sub>2</sub> PO <sub>4</sub> .....	2.7g
NaN <sub>3</sub> .....	0.3g
Ethyl Violet .....	0.5mg

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Use:** For the isolation, cultivation, and enumeration of enterococci from water and other specimens. Fecal enterococci turn the medium turbid with a purple sediment on the bottom of the tube.

### *Eubacterium* Medium

#### Composition per liter:

Pancreatic digest of casein .....	20.0g
Agar .....	15.0g
Meat extract .....	15.0g
Glucose .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	4.0g
L-Cysteine-HCl .....	0.5g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Use freshly prepared medium.

**Use:** For the cultivation of *Eubacterium* species.

### *Eubacterium oxidoreducens* Medium

#### Composition per 1001.0mL:

Solution A .....	890.0mL
Solution B .....	100.0mL
Solution C .....	10.0mL
Solution D .....	1.0mL

pH 7.0–7.2 at 25°C

#### Solution A:

##### Composition per 890.0mL:

Crotonic acid .....	5.0g
Yeast extract .....	2.0g
Resazurin .....	1.0mg
Mineral solution .....	50.0mL
Vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Adjust pH to 6.9. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min. Distribute 8.9mL into anaerobic tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Mineral Solution:

##### Composition per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	6.6g
NaCl .....	8.0g
NH <sub>4</sub> Cl .....	8.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	6.2mg
Nicotinic acid .....	2.5mg
p-Aminobenzoic acid .....	1.25mg
Thiamine-HCl .....	1.25mg
Pantothenic acid .....	0.62mg
Biotin .....	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly.

ly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

#### Solution B:

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution C:

**Composition** per 10.0mL:

L-Cysteine ..... 0.24g

**Preparation of Solution C:** Add L-cysteine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Solution D:

**Composition** per 1.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 78.0mg

**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To each tube containing 8.9mL of sterile solution A, add (using a syringe) 1.0mL of sterile solution B, 0.1mL of sterile solution C, and 0.01mL of sterile solution D.

**Use:** For the cultivation and maintenance of *Eubacterium oxidoreducans*.

### *Exiguobacterium Medium*

**Composition** per liter:

Beef extract ..... 10.0g

Peptone ..... 10.0g

NaCl ..... 5.0g

Glucose ..... 5.0g

Yeast extract ..... 3.0g

pH 8.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Exiguobacterium aurantiacum*.

### Extracted Hay Medium

**Composition:**

Hay or grass ..... 50.0g

**Preparation of Medium:** Add hay or grass to 1.0L of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 30 min. Rinse with cold water twice. Add 1.0L of distilled/deionized water, boil 30 min and rinse. Repeat this process at least 5 times. Dry the extracted hay or grass. Add 10–30 blades of extracted hay or grass to a large test tube. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Beggiatoa* species and myxotrophic *Thiothrix* species.

### EYGA Agar

**Composition** per liter:

Agar ..... 12.0g

K<sub>2</sub>HPO<sub>4</sub> ..... 1.1g

Glucose ..... 1.0g

Yeast extract ..... 1.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.86g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.5g

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

NaCl ..... 0.1g

CaCl<sub>2</sub> ..... 0.025g

Vitamin B<sub>12</sub> ..... 2.0µg

EDTA/trace elements mix ..... 3.0mL

pH 6.8 ± 0.2 at 25°C

#### EDTA/Trace Elements Mix:

**Composition** per 600.0mL:

EDTA ..... 5.0g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.2g

MnSO<sub>4</sub>·4H<sub>2</sub>O ..... 0.57g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.50g

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.161g

CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.157g

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.151g

**Preparation of EDTA/Trace Elements Mix:** Add components to distilled/deionized water and bring volume to 600.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Arthrobacter* species.

### Fay and Barry Medium

**Composition** per liter:

Amino acid ..... 10.0g

Peptone ..... 5.0g

Yeast extract ..... 3.0g

Bromocresol Purple solution ..... 5.0mL

pH 5.5 ± 0.2 at 25°C

#### Bromocresol Purple Solution:

**Composition** per 100.0mL:

Bromocresol Purple ..... 0.2g

Ethanol ..... 50.0mL

**Preparation of Bromocresol Purple Solution:** Add Bromocresol Purple to 50.0mL of absolute ethanol. Add distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. The amino acid may be L-arginine, L-ornithine, or L-lysine, depending on which amino acid decarboxylase activity is being measured. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the determination of decarboxylase activities of *Aeromonas* species.

### FB Medium (DSMZ Medium 980)

**Composition** per liter:

NH<sub>4</sub>Cl ..... 0.54g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.14g

Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	10.0mL
Yeast extract solution .....	10.0mL
Na-crotonate solution .....	10.0mL
Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	10.0mL
Trace elements solution SL-9 .....	1.0mL
Selenite-tungstate solution .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

**Na-Crotonate Solution:****Composition per 10.0mL:**

Na-crotonate .....	0.86g
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**Preparation of Na-Crotonate Solution:** Add Na-crotonate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.25g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-9:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution SL-9:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Yeast Extract Solution:****Composition per 10.0mL:**

Yeast extract .....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except Na-crotonate solution, yeast extract solution, cysteine solution, vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute to anaerobe tubes or bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium, 10.0mL sterile cysteine solution, 10.0mL sterile Na-crotonate solution, 10.0mL sterile vitamin solution, 10.0mL sterile yeast extract solution, 10.0mL sterile NaHCO<sub>3</sub> solution, and 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Sporotomaculum syntrophicum*.

**FC Agar**  
**(Fecal Coliform Agar)**  
**(m-FC Agar)**  
**(m-Fecal Coliform Agar)**

**Composition per liter:**

Agar .....	15.0g
Lactose .....	12.5g
NaCl .....	5.0g
Proteose peptone No. 3 .....	5.0g
Yeast extract .....	3.0g
Bile salts .....	1.5g
Aniline Blue .....	0.1g
Rosolic acid solution .....	10.0mL

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Rosolic Acid Solution:****Composition per 100.0mL:**

Rosolic acid .....	1.0g
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**Preparation of Rosolic Acid Solution:** Add rosolic acid to 0.2N NaOH and bring volume to 100.0L. Mix thoroughly.

**Preparation of Medium:** Add 10.0mL rosolic acid solution to 950.0mL distilled/deionized water. Mix thoroughly. Add other components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling with frequent mixing. Do not autoclave. Pour into sterile Petri dishes or leave in tubes.



**Use:** For the cultivation of fecal coliform bacteria from waters and the enumeration of coliform bacteria using the membrane filtration method.

**FC Agar**  
**(Fecal Coliform Agar)**  
**(m-FC Agar)**  
**(m-Fecal Coliform Agar)**

**Composition per liter:**

Agar .....	15.0g
Lactose .....	12.5g
Tryptose .....	10.0g
NaCl .....	5.0g
Proteose peptone No. 3 .....	5.0g
Yeast extract .....	3.0g
Bile salts .....	1.5g
Aniline Blue .....	0.1g
Rosolic acid solution .....	10.0mL

pH 7.4 ± 0.2 at 25°C

**Rosolic Acid Solution:**

**Composition per 100.0mL:**

Rosolic acid .....	1.0g
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**Preparation of Rosolic Acid Solution:** Add rosolic acid to 0.2N NaOH and bring volume to 100.0L. Mix thoroughly.

**Preparation of Medium:** Add 10.0mL rosolic acid solution to 950.0mL of distilled/deionized water. Mix thoroughly. Add other components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling with frequent mixing. Do not autoclave. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of fecal coliform bacteria from waters and the enumeration of coliform bacteria using the membrane filtration method.

**FC Broth**  
**(Fecal Coliform Broth)**  
**(m-FC Broth)**  
**(m-Fecal Coliform Broth)**

**Composition per liter:**

Lactose .....	12.5g
Tryptose .....	10.0g
NaCl .....	5.0g
Proteose peptone No. 3 .....	5.0g
Yeast extract .....	3.0g
Bile salts .....	1.5g
Aniline Blue .....	0.1g
Rosolic acid solution .....	10.0mL

pH 7.4 ± 0.2 at 25°C

**Rosolic Acid Solution:**

**Composition per 100.0mL:**

Rosolic acid .....	1.0g
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**Preparation of Rosolic Acid Solution:** Add rosolic acid to 0.2N NaOH and bring volume to 100.0L. Mix thoroughly.

**Preparation of Medium:** Add 10.0mL of rosolic acid solution to 950.0mL of distilled/deionized water. Mix thoroughly. Add other components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling with frequent mixing. Do not autoclave. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of fecal coliform bacteria from waters and the enumeration of coliform bacteria using the membrane filtration method.

**FC Broth**  
**(Fecal Coliform Broth)**  
**(m-FC Broth)**  
**(m-Fecal Coliform Broth)**

**Composition per liter:**

Lactose .....	12.5g
NaCl .....	5.0g
Proteose peptone No. 3 .....	5.0g
Yeast extract .....	3.0g
Bile salts .....	1.5g
Aniline Blue .....	0.1g
Rosolic acid solution .....	10.0mL

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Rosolic Acid Solution:**

**Composition per 100.0mL:**

Rosolic acid .....	1.0g
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**Preparation of Rosolic Acid Solution:** Add rosolic acid to 0.2N NaOH and bring volume to 100.0L. Mix thoroughly.

**Preparation of Medium:** Add 10.0mL of rosolic acid solution to 950.0mL of distilled/deionized water. Mix thoroughly. Add other components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling with frequent mixing. Do not autoclave. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of fecal coliform bacteria from waters and the enumeration of coliform bacteria using the membrane filtration method.

**Fe(III) Lactate Nutrient Agar**

**Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g
Fe(III)-lactate solution .....	25.0mL

pH 7.2 ± 0.2 at 25°C

**Fe(III)-Lactate Solution:**

**Composition per 30.0mL:**

FeCl <sub>3</sub> ·6H <sub>2</sub> O solution .....	20.0mL
Sodium lactate solution .....	10.0mL

**Preparation of Fe(III)-Lactate Solution:** Aseptically combine the component solutions. Mix thoroughly.

**FeCl<sub>3</sub>·6H<sub>2</sub>O Solution:**

**Composition per 100.0mL:**

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	5.0g
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**Preparation of FeCl<sub>3</sub>·6H<sub>2</sub>O Solution:** Add FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Sodium Lactate Solution:**

**Composition per 100.0mL:**

Sodium lactate .....	5.0g
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**Preparation of Sodium Lactate Solution:** Add sodium lactate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Fe(III)-lactate solution, to distilled/deionized water and bring volume to 975.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure—

121°C. Cool to 50°–55°C. Aseptically add 25.0mL of filter-sterilized Fe(III)-lactate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Shewanella putrefaciens*.

### Fecal Coliform Agar, Modified (m-Fecal Coliform Agar, Modified) (FCIC)

**Composition** per liter:

Agar .....	15.0g
Inositol .....	10.0g
Tryptose .....	10.0g
Proteose peptone No. 3 .....	5.0g
NaCl .....	5.0g
Yeast extract .....	3.0g
Bile salts No. 3 .....	1.5g
Aniline Blue .....	0.1g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 50°C. Adjust pH to 7.4. Pour into sterile Petri dishes in 20.0mL volumes. Allow surface of plates to dry before using.

**Use:** For the isolation, cultivation and enumeration of *Klebsiella* species using the membrane filter method.

### Fecal Coliform Agar, Modified

**Composition** per liter:

Agar .....	15.0g
Lactose .....	12.5g
Tryptose .....	10.0g
Proteose peptone No. 3 .....	5.0g
NaCl .....	5.0g
Yeast extract .....	3.0g
Bile salts No. 3 .....	1.5g
Aniline Blue .....	0.1g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 50°C. Adjust pH to 7.4. Pour into sterile Petri dishes in 20.0mL volumes. Allow surface of plates to dry before using.

**Use:** For the isolation, cultivation, and identification of stressed fecal coliform microorganisms based on their ability to ferment lactose. Lactose-fermenting bacteria turn the medium blue.

### Feodorov Medium

**Composition** per liter:

Mannitol or glucose .....	20.0g
Marine salts mixture .....	18.0g
CaCO <sub>3</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> .....	0.3g
CaHPO <sub>4</sub> .....	0.2g
K <sub>2</sub> SO <sub>4</sub> .....	0.2g
FeCl <sub>3</sub> .....	0.1g
Trace elements solution .....	1.0mL

**Trace Elements Solution:**

**Composition** per 100.0mL:

H <sub>3</sub> BO <sub>3</sub> .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> · 4H <sub>2</sub> O .....	0.5g
KI .....	0.05g
NaBr .....	0.05g

Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> · 18H <sub>2</sub> O .....	0.03g
ZnSO <sub>4</sub> .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Azotobacter vinelandii*.

### Ferric Citrate Medium

**Composition** per liter:

Ferric citrate .....	13.7g
Sodium lactate (60% solution) .....	5.6g
NaHCO <sub>3</sub> .....	2.5g
NH <sub>4</sub> Cl .....	1.5g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.6g
KCl .....	0.1g
Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Wolfe's Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium D-(+)-pantothenate .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Wolfe's Mineral Solution:**

**Composition** per liter:

MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> · H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> · 6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> · 12H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> · 5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components sequentially. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add ferric citrate to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling. Continue boiling until ferric citrate is dissolved. Cool to room temperature. Adjust to pH 6.6 with 10N NaOH. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute

into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Final pH should be 7.0.

**Use:** For the cultivation of *Aeromonas encheleia* and *Shewanella alga*.

***Ferroglobus placidus* Medium  
(DSMZ Medium 730)**

**Composition per 1020mL:**

NaCl .....	18.0g
NaHCO <sub>3</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.3g
KNO <sub>3</sub> .....	1.0g
KCl .....	0.34g
NH <sub>4</sub> Cl .....	0.24g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.14g
Resazurin .....	0.5mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Na-pyruvate solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Na-pyruvate Solution:**

**Composition per 10.0mL:**

Na-pyruvate .....	1.0g
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**Preparation of Na-pyruvate Solution:** Add Na-pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix

thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, vitamin solution, and Na-pyruvate solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Flush medium with N<sub>2</sub> for 20 min. Adjust medium pH to 7.0 with 4N H<sub>2</sub>SO<sub>4</sub>. Add 10.0mL of Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Readjust the medium pH to 7.0 with H<sub>2</sub>SO<sub>4</sub>, while flushing the gas phase only with N<sub>2</sub>. Dispense 10 mL volumes into 100 mL serum bottles with rubber stoppers under N<sub>2</sub>. Replace gas phase by 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture and finally pressurize the bottles to 2 bar gas overpressure. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically inject via syringe 0.1mL vitamin mix and 0.1mL Na-pyruvate solution into each tube containing 10.0mL of the autoclaved medium.

**Use:** For the cultivation of *Ferroglobus placidus*.

***Ferroplasma acidiphilum* Medium  
(DSMZ Medium 874)**

**Composition per 1001.6mL:**

Solution A .....	950.0mL
Solution B .....	10.0mL
Solution C .....	1.6mL

pH 1.7 ± 0.2 at 25°C

**Solution A:**

**Composition per 950mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
KCl .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly.

**Solution B:**

**Composition per 50mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	25.0g
H <sub>2</sub> SO <sub>4</sub> , 1N .....	40.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**Solution C:**

**Composition per 10mL:**

Yeast extract .....	1.0g
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**Preparation of Solution C:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add 50.0mL solution B to 950.0mL solution A. Mix thoroughly. Adjust pH to 1.6–1.8 with H<sub>2</sub>SO<sub>4</sub>. Filter sterilize. Aseptically add 1.6 mL of sterile solution C. Pour into sterile Petri dishes or leave in tubes. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Ferropasma acidiphilum* (*Ferriplasma acidiphilum*).

### Ferrous Sulfide Agar

**Composition** per 1200.0mL:

Agar layer .....	1.0L
Liquid overlay .....	200.0mL

### Agar Layer:

**Composition** per liter:

Agar .....	30.0g
FeS washed precipitate suspension .....	500.0mL

**Preparation of Agar Layer:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Heat FeS washed precipitate suspension to 45°–50°C. Mix thoroughly. Aseptically add 500.0mL of sterile FeS washed precipitate suspension to 500.0mL of sterile agar at 45°–50°C. Mix thoroughly.

### FeS Washed Precipitate Suspension:

**Composition** per 500.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	78.4g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	15.6g

**Preparation of FeS Washed Precipitate Suspension:** Add Na<sub>2</sub>S·9H<sub>2</sub>O and Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> to 500.0mL boiling distilled/deionized water. Let precipitate settle from the hot solution in a completely filled and stoppered bottle. Wash precipitate four times by decanting supernatant and replacing each time with 500.0mL of boiling distilled/deionized water. Store FeS washed precipitate suspension in a completely filled 500.0mL glass-stoppered bottle.

### Liquid Overlay:

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.1g

**Preparation of Liquid Overlay:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically bubble 100% CO<sub>2</sub> for 15 sec.

**Preparation of Medium:** Aseptically distribute agar layer into sterile tubes in 10.0mL volumes. Allow tubes to cool in a slanted position. Aseptically add 2.0mL of sterile liquid overlay to each tube.

**Use:** For the enumeration, enrichment, and isolation of iron and sulfur bacteria, including *Gallionella ferruginea*.

### Fervidobacterium islandicum Medium

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> B <sub>4</sub> ·10H <sub>2</sub> O .....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8mg
Resazurin .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

Glucose solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

### Glucose Solution:

**Composition** per 20.0mL:

Glucose .....	2.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile glucose solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Fervidobacterium islandicum*.

### Fervidobacterium Medium

**Composition** per liter:

Pancreatic digest of casein .....	10.0g
Glucose .....	5.0g
Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution .....	9.0mL
Wolfe's vitamin solution .....	5.0mL
Resazurin (0.2% solution) .....	1.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (10% solution) .....	0.03mL
pH 7.0 ± 0.1 at 25°C	

### Trace Elements Solution:

**Composition** per liter:

Nitrilotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Filter sterilize. Maintain under an atmosphere of 100% N<sub>2</sub>.

### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	0.01g
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg

Nicotinic acid.....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Maintain under an atmosphere of 100% N<sub>2</sub>.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Maintain under an atmosphere of 100% N<sub>2</sub>.

**Preparation of Medium:** Add components, except sodium sulfide solution, trace elements solution, and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 976.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under an atmosphere of 100% N<sub>2</sub>. Aseptically add 9.0mL of trace elements solution and 5.0mL of Wolfe's vitamin solution under an atmosphere of 100% N<sub>2</sub>. Mix thoroughly. Aseptically distribute into sterile tubes or flasks under an atmosphere of 100% N<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O solution just prior to use to a concentration 0.1%.

**Use:** For the cultivation and maintenance of *Clostridium* species, *Fervidobacterium nodosum*, *Fervidobacterium islandicum*, and *Thermoanaerobium brockii*.

### F-G Agar (Feeley-Gorman Agar)

**Composition per liter:**

Casein, acid hydrolyzed.....	17.5g
Agar .....	17.0g
Beef extract.....	3.0g
Starch .....	1.5g
L-Cysteine solution .....	10.0mL
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> solution .....	10.0mL

pH 6.9 ± 0.05 at 25°C

#### L-Cysteine Solution:

**Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.4g
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**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:

**Composition per 10.0mL:**

Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> .....	0.25g
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**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:** Add Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine solution and Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of L-cysteine solution. Mix thoroughly. Aseptically add 10.0mL of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution. Mix thor-

oughly. Adjust pH to 6.9. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Legionella pneumophila*.

### F-G Agar with Selenium (Feeley-Gorman Agar with Selenium)

**Composition per liter:**

Casein, acid hydrolyzed .....	17.5g
Agar.....	17.0g
Beef extract .....	3.0g
Starch.....	1.5g
L-Cysteine solution.....	10.0mL
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O solution.....	10.0mL

pH 6.9 ± 0.05 at 25°C

#### L-Cysteine Solution:

**Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.4g
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**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:

**Composition per 10.0mL:**

Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> .....	0.25g
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**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:** Add Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.01g
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**Preparation of Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O Solution:** Add 0.1g of Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components—except L-cysteine solution, Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution, and Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O solution—to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile L-cysteine solution. Mix thoroughly. Aseptically add 10.0mL of sterile Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> solution and 10.0mL of sterile Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 6.9. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Legionella pneumophila*.

### FGTC Agar

**Composition per liter:**

Pancreatic digest of casein .....	15.0g
Agar .....	15.0g
Papaic digest of soybean meal .....	5.0g
NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.0g
Amylose Azure.....	3.0g
Galactose .....	1.0g
Thallous acetate.....	0.5g
MUG (4-Methylumbelliferyl- α-D-galactoside).....	0.1g
NaHCO <sub>3</sub> solution.....	20.0mL
Gentamicin solution .....	2.5mL
Tween 80 .....	0.75mL

pH 7.3 ± 0.2 at 25°C

# **Gentamicin Solution:**

## **Composition** per 10.0mL:

Gentamicin..... 0.01g

**Preparation of Gentamicin Solution:** Add gentamicin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

# **NaHCO<sub>3</sub> Solution:**

## **Composition** per 20.0mL:

NaHCO<sub>3</sub>..... 2.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Use freshly prepared solution.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the cultivation, differentiation, and enumeration of *Enterococcus* species based on starch hydrolysis and production of fluorescence. Bacteria that hydrolyze starch, such as *Streptococcus bovis*, appear as colonies surrounded by a clear zone. Bacteria that produce fluorescence, such as *Streptococcus bovis* and *Enterococcus faecium*, appear as colonies surrounded by a zone of bluish fluorescence when viewed under a long-wave UV lamp. Other bacteria, such as *Enterococcus faecalis* and *Enterococcus avium*, do not hydrolyze starch or produce fluorescence.

# **Fish Peptone Broth**

## **Composition** per liter:

Maltose..... 5.0g

NaCl..... 5.0g

Peptone..... 5.0g

Pancreatic digest of casein..... 5.0g

Yeast extract..... 5.0g

Trout tissue extract solution..... 50.0mL

pH 7.0 ± 0.2 at 25°C

# **Trout Tissue Extract Solution:**

## **Composition** per liter:

Fish (brook trout)..... 500.0g

Pepsin..... 1.0g

HCl, concentrated..... 15.0mL

**Preparation of Trout Tissue Extract:** Add 1.0L of distilled/deionized water to brook trout and blend for 20–30 min. Add 1.0g of pepsin and 15.0mL of concentrated HCl to digest the trout proteins. Incubate for 12 hr at 45°C. Adjust pH to 7.0. Allow solids to settle. Filter sterilize. Do not autoclave. Store at 5°C.

**Preparation of Medium:** Add components, except trout tissue extract, to distilled/deionized water and bring volume to 950.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 10 psi pressure–118°C. Cool to 45°–50°C. Aseptically add 50.0mL of sterile trout tissue extract. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Aeromonas salmonicida*.

# ***Flavobacterium aquatile* Medium (DSMZ Medium 102)**

## **Composition** per liter:

Agar..... 15.0g

Na-caseinate..... 2.0g

Proteose peptone..... 1.0g

Yeast extract..... 0.5g

K<sub>2</sub>HPO<sub>4</sub>..... 0.5g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Flavobacterium aquatile*.

# ***Flavobacterium* M1 Agar**

## **Composition** per liter:

Agar..... 15.0g

Proteose peptone..... 5.0g

NaCl..... 3.0g

Beef extract..... 2.0g

Yeast extract..... 1.0g

pH 7.0–7.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flavobacterium indoltheticum*.

# ***Flavobacterium* Medium**

## **Composition** per liter:

Na<sub>2</sub>SO<sub>4</sub>..... 1.0g

Pancreatic digest of casein..... 1.0g

Yeast extract..... 1.0g

pH 6.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Flavobacterium acidurans*.

# ***Flavobacterium* Medium (ATCC Medium 65)**

## **Composition** per liter:

Agar..... 12.0g

Sodium caseinate..... 2.0g

Peptone..... 1.0g

K<sub>2</sub>HPO<sub>4</sub>..... 0.5g

Yeast extract..... 0.5g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flavobacterium aquatile*.

# ***Flavobacterium* Medium (ATCC Medium 1687)**

## **Composition** per liter:

Sodium glutamate..... 4.0g

K<sub>2</sub>HPO<sub>4</sub>..... 0.65g

NaNO<sub>3</sub>..... 0.5g

KH <sub>2</sub> PO <sub>4</sub> .....	0.19g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> solution.....	2.0mL
pH 7.4 ± 0.2 at 25°C	

**FeSO<sub>4</sub> Solution:****Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.03g
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**Preparation of FeSO<sub>4</sub> Solution:** Add FeSO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except FeSO<sub>4</sub> solution, to distilled/deionized water and bring volume to 998.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 2.0mL of sterile FeSO<sub>4</sub> solution. Mix thoroughly. Adjust pH to 7.4. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Flavobacterium* species.

***Flavobacterium* Medium M1****Composition** per liter:

Agar.....	12.0g
Proteose peptone.....	5.0g
NaCl.....	3.0g
Beef extract.....	2.0g
Yeast extract.....	0.2g

pH 7.2–7.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Flavobacterium* species.

***Flavobacterium* Medium with Thiamine****Composition** per liter:

Agar.....	12.0g
Sodium caseinate.....	2.0g
Peptone.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Yeast extract.....	0.5g
Thiamine·HCl.....	10.0mg

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flavobacterium aquatile* and *Flavobacterium lutescens*.

***Flavobacterium resinovorum* Agar  
(LMG Medium 216)****Composition** per liter:

Agar.....	15.0g
Lab-Lemco beef extract.....	10.0g
Peptone.....	10.0g
NaCl.....	5.0g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flavobacterium resinovorum*.

**Fletcher Medium****Composition** per liter:

Agar.....	1.5g
NaCl.....	0.5g
Peptone.....	0.3g
Beef extract.....	0.2g
Rabbit serum.....	50.0mL

pH 7.9 ± 0.1 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except rabbit serum, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile rabbit serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation, cultivation, and maintenance of cultures of *Leptospira* species.

**Fletcher Medium with Fluorouracil  
(Fluorouracil *Leptospira* Medium)****Composition** per liter:

Agar.....	1.5g
NaCl.....	0.5g
Peptone.....	0.3g
Beef extract.....	0.2g
Rabbit serum.....	50.0mL
Fluorouracil solution.....	20.0mL

pH 7.9 ± 0.1 at 25°C

**Fluorouracil Solution:****Composition** per 100.0mL:

Fluorouracil.....	10.0g
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**Preparation of Fluorouracil Solution:** Add fluorouracil to 50.0mL of distilled/deionized water. Add 1.0mL of 2N NaOH and bring volume to 100.0mL. Gently heat to 56°C for 2 hr. Adjust pH to 7.4–7.6 with NaOH. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except rabbit serum and fluorouracil solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 80.0mL of sterile rabbit serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Immediately prior to use, add 0.1mL of fluorouracil solution per 5.0mL of medium.

**Use:** For the isolation, cultivation, and maintenance of cultures of *Leptospira* species.

***Flexibacter* Agar  
(LMG Medium 60)****Composition** per liter:

Agar.....	15.0g
Casamino acids.....	1.0g
Tris buffer.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KNO <sub>3</sub> .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
Sodium-β-glycerophosphate.....	0.1g
Thiamine.....	1.0mg
Vitamin B <sub>12</sub> .....	1.0μg

Glucose solution .....	10.0mL
Vitamin solution.....	10.0mL
Trace elements solution .....	1.0mL
pH 7.5 ± 0.2 at 25°C	

**Glucose Solution:****Composition per 100.0mL:**

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.85g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.49g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
Sodium tartrate.....	1.77g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	40.4mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	26.9mg
ZnCl <sub>2</sub> .....	20.8mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	25.2mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition per 10.0mL:**

Thiamine .....	10.0mg
Vitamin B <sub>12</sub> .....	10.0µg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, glucose solution, and trace elements solution, to 979.0mL distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile vitamin solution, 10.0mL of sterile glucose solution, and 1.0mL of sterile trace elements solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Flexibacter* spp.

**Flexibacter Agar****Composition per liter:**

Agar .....	15.0g
Sodium glutamate .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Yeast extract.....	1.0g
Glucose solution .....	20.0mL

pH 7.2 ± 0.2 at 25°C

**Glucose Solution:****Composition per 20.0mL:**

Glucose .....	2.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Flexibacter elegans*.

**Flexibacter canadensis Agar****Composition per 1001.0mL:**

Agar .....	10.0g
Casamino acids.....	1.0g
Tris.....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Sodium glycerophosphate .....	0.1g
Thiamine HCl .....	1.0mg
Vitamin B <sub>12</sub> .....	1.0µg
Glucose solution .....	10.0mL
Trace elements solution SL-10.....	1.0mL

pH 7.5 ± 0.2 at 25°C

**Glucose Solution:****Composition per 10.0mL:**

Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Flexibacter canadensis*.

**Flexibacter Medium****Composition per liter:**

Agar .....	15.0g
Tris(hydroxymethyl)amino methane buffer .....	1.0g
Casamino acids.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
KNO <sub>3</sub> .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Sodium β-glycerophosphate.....	0.1g
Thiamine.....	1.0mg
Cobalamin .....	1.0µg
Glucose solution .....	10.0mL
Trace elements solution HO-LE.....	1.0mL

pH 7.5 ± 0.2 at 25°C

**Glucose Solution:****Composition per 10.0mL:**

D-Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution HO-LE:

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.85g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
Sodium tartrate .....	1.77g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.36g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.027g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
ZnCl <sub>2</sub> .....	0.020g

**Preparation of Trace Elements Solution HO-LE:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Flexibacter* species.

#### *Flexibacter* Medium (ATCC Medium 1559)

**Composition per liter:**

Solution B .....	700.0mL
Solution A .....	300.0mL

#### Solution A:

**Composition per 300.0mL:**

Pancreatic digest of casein .....	0.5g
Yeast extract .....	0.5g
Beef extract .....	0.2g
Sodium acetate .....	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Solution B:

Aged seawater .....	700.0 mL
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**Preparation of Solution B:** Allow seawater to sit for 7 days. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically add 300.0mL of sterile solution A to 700.0mL of sterile solution B at 45°–50°C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Flexibacter maritimus*.

#### *Flexibacter polymorphus* Medium (LMG 108)

**Composition per liter:**

Agar .....	15.0g
Monosodium glutamate .....	5.0g
Tryptone .....	1.0g
Casamino acids, vitamin-free .....	1.0g
Sodium glycerophosphate .....	0.1g
Vitamin B <sub>12</sub> .....	1.0μg
Trace elements solution HO-LE .....	1.0mL
Seawater, filtered, aged .....	1.0L

#### Trace Elements Solution HO-LE:

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.85g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
Sodium tartrate .....	1.77g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.36g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.027g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
ZnCl <sub>2</sub> .....	0.02g

#### Preparation of Trace Elements Solution HO-LE:

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Flexibacter polymorphus*.

#### *Flexibacterium* Medium

**Composition per 1060.0mL:**

Yeast extract .....	1.0g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.02g
Seawater, filtered .....	1.0L
Glucose solution .....	50.0mL
Trace elements .....	10.0mL

pH 7.0 ± 0.2 at 25°C

#### Glucose Solution:

**Composition per 50.0mL:**

Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Trace Elements:

**Composition per liter:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3mg
H <sub>3</sub> BO <sub>3</sub> .....	0.1mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.1mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components, except glucose solution. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Flexibacter litoralis* and *Flexibacter marinus*.

#### *Flexibacterium* Medium

**Composition per 1050.0mL:**

Tris(hydroxymethyl)aminomethane buffer .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KCl .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Sodium glycerophosphate .....	0.1g

NaNO <sub>3</sub> .....	0.1g
Cobalamin.....	1.0μg
Glucose solution.....	50.0mL
Trace elements.....	10.0mL
pH 7.5 ± 0.2 at 25°C	

**Glucose Solution:****Composition per 50.0mL:**

Glucose.....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Trace Elements:****Composition per liter:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.3mg
H <sub>3</sub> BO <sub>3</sub> .....	0.1mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.1mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1mg

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Saprospira thermalis*, *Flexibacter elegans*, and *Flexibacter rubrum*.

**Flexithrix Marine Agar  
(LMG Medium 61)**

**Composition per liter:**

Agar.....	15.0g
Tryptone.....	5.0g
Yeast extract.....	5.0g
Tris buffer.....	1.0g
KNO <sub>3</sub> .....	0.5g
Sodium-beta-glycerophosphate.....	0.1g
Trace elements solution.....	1.0mL
pH 7.4 ± 0.2 at 25°C	

**Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.85g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.49g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8g
Sodium tartrate.....	1.77g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	40.4mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	26.9mg
ZnCl <sub>2</sub> .....	20.8mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	25.2mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to filtered aged seawater and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Flexithrix dorotheae*.

**FIGlyM Medium  
(DSMZ Medium 298b)**

**Composition per liter:**

NaCl.....	1.0g
KCl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
NaHCO <sub>3</sub> solution.....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Na-glycolate solution.....	10.0mL
L-Cysteine solution.....	10.0mL
Na-acetate solution.....	7.0mL
Trace elements solution SL-10.....	1.0mL
Vitamin solution.....	0.5mL
pH 7.2 ± 0.2 at 25°C	

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na-Acetate Solution:****Composition per 10.0mL:**

Na-acetate.....	0.25g
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**Preparation of Na-Acetate Solution:** Add Na-acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na-Glycolate Solution:****Composition per 10.0mL:**

Na-glycolate.....	1.0g
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**Preparation of Na-Glycolate Solution:** Add Na-glycolate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**L-Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
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**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition per liter:**

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
Folic Acid .....	30.0mg
D(+)-Biotin .....	20.0mg
$\alpha$ -Lipoic acid .....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, L-cysteine solution, Na-acetate solution, Na-glycolate solution, vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 979.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL L-cysteine solution, 7.0mL Na-acetate solution, 10.0mL Na-glycolate solution, 1.0mL trace elements solution SL-10, and 0.5mL vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% N<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Syntrophobotulus* spp.

**Flo Agar****Composition per liter:**

Agar .....	14.0g
Pancreatic digest of casein .....	10.0g
Peptic digest of animal tissue .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For cultivation of fluorescent *Pseudomonas* species.

**Fluorescent Pectolytic Agar  
(FPA Medium)****Composition per liter:**

Proteose peptone No. 3 .....	20.0g
Agar .....	15.0g
Pectin .....	5.0g

K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.73g
Antibiotic solution .....	10.0mL

**Antibiotic Solution:****Composition per 10.0mL:**

Cycloheximide .....	0.075g
Novobiocin .....	0.045g
Penicillin G .....	75,000U
Ethanol .....	1.0mL

**Preparation of Antibiotic Solution:** Add components to 1.0mL of ethanol. Mix thoroughly. Let stand for 30 min. Bring volume to 10.0mL with distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except antibiotic solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the cultivation of fluorescent *Pseudomonas* species that are pectinolytic.

**FN Medium****(Fluorescence Denitrification Medium)****Composition per liter:**

Agar .....	15.0g
Proteose peptone No. 3 .....	10.0g
KNO <sub>3</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g
NaNO <sub>2</sub> .....	0.5g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the differentiation of pseudomonads from other nonfermentative bacilli. Denitrification from nitrate or nitrite is indicated by the formation of gas bubbles in the solid medium. *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, and *Pseudomonas denitrificans* are positive for denitrification. Fluorescein production is indicated by fluorescence under UV light. *Pseudomonas aeruginosa* is positive for fluorescein production; *Pseudomonas denitrificans* does not produce fluorescein.

**FPA Medium****Composition per liter:**

Proteose peptone No. 3 .....	20.0g
Agar .....	15.0g
Pectin, citrus .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g
Antibiotic solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Antibiotic Solution:****Composition per 10.0mL:**

Cycloheximide .....	0.075g
Novobiocin .....	0.045g
Penicillin G .....	75,000U

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except agar and antibiotic solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 7.0. Add agar. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Pseudomonas* species that cause soft rot.

#### Frankia Agar

**Composition** per liter:

Starch, soluble.....	20.0g
Agar .....	15.0g
Glucose .....	10.0g
N-Z-Amine.....	5.0g
Yeast extract.....	5.0g
CaCO <sub>3</sub> .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Frankia alni*.

#### Frankia Isolation Medium

**Composition** per liter:

Sucrose.....	40.0g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O.....	0.242g
KNO <sub>3</sub> .....	0.085g
KCl.....	0.061g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.042g
KH <sub>2</sub> PO <sub>4</sub> .....	0.02g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	4.5mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	2.5mg
H <sub>3</sub> BO <sub>3</sub> .....	1.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.5mg
Nicotinic acid.....	0.5mg
Pyridoxine-HCl.....	0.5mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.25mg
Thiamine-HCl.....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.04mg
Mannitol solution.....	10.0mL
Supplement solution.....	10.0mL

pH 5.5 ± 0.2 at 25°C

#### Mannitol Solution:

**Composition** per 100.0mL:

Mannitol.....	11.84g
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**Preparation of Mannitol Solution:** Add mannitol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Supplement Solution:

**Composition** per 10.0mL:

L-Glutamic acid.....	0.185g
L-Arginine.....	0.174g
L-Glutamine.....	0.146g
L-Aspartic acid.....	0.133g
L-Asparagine.....	0.132g
Glycine.....	0.075g
Urea.....	0.06g
Naphthaloneacetic acid.....	2.0mg
Zeatin.....	1.0μg

**Preparation of Supplement Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except mannitol solution and supplement solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 5.5. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile mannitol solution and 10.0mL of sterile supplement solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Frankia* species from root nodules.

#### Fraser Broth

**Composition** per liter:

NaCl.....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	12.0g
Beef Extract.....	5.0g
Proteose peptone.....	5.0g
Pancreatic digest of casein.....	5.0g
Yeast extract.....	5.0g
LiCl.....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.35g
Esculin.....	1.0g
Fraser supplement solution.....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

#### Fraser Supplement Solution:

**Composition** per 10.0mL:

Ferric ammonium citrate.....	0.5g
Acriflavine-HCl.....	0.25g
Nalidixic acid.....	0.1g
Ethanol.....	5.0mL

**Preparation of Fraser Supplement Solution Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Fraser supplement solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile Fraser supplement solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation of *Listeria* species from environmental species.

#### Fraser Secondary Enrichment Broth

**Composition** per liter:

NaCl.....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	12.0g
Beef extract.....	5.0g
Proteose peptone.....	5.0g
Pancreatic digest of casein.....	5.0g
Yeast extract.....	5.0g
LiCl.....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.35g
Esculin.....	1.0g
Acriflavin solution.....	10.0mL
Ferric ammonium citrate solution.....	10.0mL
Nalidixic acid solution.....	1.0mL

**Ferric Ammonium Citrate Solution:****Composition** per 10.0mL:

Ferric ammonium citrate..... 0.5g

**Preparation of Ferric Ammonium Citrate Solution:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Acriflavin Solution:****Composition** per 10.0mL:

Acriflavin..... 0.025g

**Preparation of Acriflavin Solution:** Add acriflavin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Nalidixic Acid Solution:****Composition** per 10.0mL:

Nalidixic acid..... 0.04g

NaOH (0.1*N* solution)..... 10.0mL

**Preparation of Nalidixic Acid Solution:** Add nalidixic acid to 10.0mL of NaOH solution. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except acriflavin solution and ferric ammonium citrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 12 min at 15 psi pressure–121°C. Cool rapidly to 25°C. Immediately prior to inoculation, aseptically add 0.1mL of sterile acriflavin solution and 0.1mL of ferric ammonium citrate solution to each tube. Mix thoroughly.

**Use:** For the isolation, cultivation, and enrichment of *Listeria monocytogenes* from environmental specimens based on esculin hydrolysis. Bacteria that hydrolyze esculin appear as black colonies.

**Freshwater Ameba Medium****Composition** per liter:

Agar ..... 10.0g

Malt extract ..... 0.1g

Yeast extract..... 0.1g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Acanthamoeba astronyxis*, *Acanthamoeba castellanii*, *Acanthamoeba griffini*, *Acanthamoeba pearcei*, *Acanthamoeba polyphaga*, *Acanthamoeba rhysodes*, *Acanthamoeba stevensoni*, *Acanthamoeba tubiashi*, *Capsellina* species, *Cochliopodium actinophora*, *Cochliopodium bilimbosum*, *Hartmannella limax*, *Hartmannella vermiformis*, *Naegleria fowleri*, *Naegleria gruberi*, *Naegleria lovaniensis*, *Naegleria minor*, *Naegleria thorntoni*, *Paraflagellula reniformis*, *Protacanthamoeba caledonica*, *Rosculus* species, *Saccamoeba limax*, *Tetramitus rostratus*, *Vahlkampfia inornata*, and *Vanella miroides*.

**FSM Selective Medium****Composition** per liter:

Agar ..... 18.0g

Peptone..... 10.0g

Glucose..... 4.0g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>..... 1.32g

K<sub>2</sub>HPO<sub>4</sub>..... 1.18g

Casamino acids..... 1.0g

Yeast extract ..... 1.0g

KH<sub>2</sub>PO<sub>4</sub>..... 0.44g

MgSO<sub>4</sub>·7H<sub>2</sub>O..... 0.2g

FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·5H<sub>2</sub>O..... 3.0mg

Citric acid ..... 1.9mg

ZnSO<sub>4</sub>·7H<sub>2</sub>O..... 1.6mg

MnSO<sub>4</sub>·H<sub>2</sub>O..... 1.5mg

2,3,5-Triphenyltetrazolium-HCl solution ..... 10.0mL

Benomyl solution ..... 10.0mL

Polymyxin B solution..... 10.0mL

Chloroneb solution ..... 10.0mL

Dichloran solution ..... 10.0mL

Bacitracin solution..... 10.0mL

Cycloheximide solution..... 10.0mL

Pentachloronitrobenzene solution ..... 10.0mL

Pimaricin solution ..... 10.0mL

Tyrothricin solution ..... 10.0mL

Vancomycin solution ..... 10.0mL

Chloromycetin solution ..... 10.0mL

Penicillin G solution..... 10.0mL

**2,3,5-Triphenyltetrazolium-HCl Solution:****Composition** per 10.0mL:

2,3,5-Triphenyltetrazolium-HCl ..... 0.5mg

**Preparation of 2,3,5-Triphenyltetrazolium-HCl Solution:** Add 2,3,5-triphenyltetrazolium-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 7 min at 15 psi pressure–121°C.

**Benomyl Solution:****Composition** per 10.0mL:

Benomyl ..... 0.5mg

**Preparation of Benomyl Solution:** Add benomyl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Polymyxin B Solution:****Composition** per 10.0mL:

Polymyxin B..... 0.1mg

**Preparation of Polymyxin B Solution:** Add polymyxin B to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Chloroneb Solution:****Composition** per 10.0mL:

Chloroneb ..... 0.1mg

**Preparation of Chloroneb Solution:** Add chloroneb to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Dichloran Solution:****Composition** per 10.0mL:

Dichloran..... 0.1mg

**Preparation of Dichloran Solution:** Add dichloran to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Bacitracin Solution:****Composition** per 10.0mL:

Bacitracin ..... 0.05mg

**Preparation of Bacitracin Solution:** Add bacitracin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Cycloheximide Solution:****Composition** per 10.0mL:

Cycloheximide ..... 0.05mg

**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Pentachloronitrobenzene Solution:**

**Composition per 10.0mL:**  
Pentachloronitrobenzene..... 0.03mg

**Preparation of Pentachloronitrobenzene Solution:** Add pentachloronitrobenzene to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Pimaricin Solution:**

**Composition per 10.0mL:**  
Pimaricin..... 0.02mg

**Preparation of Pimaricin Solution:** Add pimaricin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Tyrosine Solution:**

**Composition per 10.0mL:**  
Tyrosine..... 0.02mg

**Preparation of Tyrosine Solution:** Add tyrosine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Vancomycin Solution:**

**Composition per 10.0mL:**  
Vancomycin ..... 0.01mg

**Preparation of Vancomycin Solution:** Add vancomycin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Chloromycetin Solution:**

**Composition per 10.0mL:**  
Chloromycetin ..... 5.0µg

**Preparation of Chloromycetin Solution:** Add chloromycetin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Penicillin G Solution:**

**Composition per 10.0mL:**  
Penicillin G ..... 1.0µg

**Preparation of Penicillin G Solution:** Add penicillin G to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components—except 2,3,5-triphenyltetrazolium chloride solution, benomyl solution, polymyxin B solution, chloroneb solution, dichloran solution, bacitracin solution, cycloheximide solution, pentachloronitrobenzene solution, pimaricin solution, tyrosine solution, vancomycin solution, chloromycetin solution, and penicillin G solution—to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL each of sterile 2,3,5-triphenyltetrazolium chloride solution, benomyl solution, polymyxin B solution, chloroneb solution, dichloran solution, bacitracin solution, cycloheximide solution, pentachloronitrobenzene solution, pimaricin solution, tyrosine solution, vancomycin solution, chloromycetin solution, and penicillin G solution. Mix thoroughly. Pour into sterile Petri dishes. Dry plates for 24 hr at 30°C.

**Use:** For the isolation and cultivation of *Pseudomonas solanacearum* from soil.

**FTX Broth**

**Composition per 1001.0mL:**

Sodium glutamate.....	10.0g
Glucose.....	2.0g
Tris.....	2.0g
Sodium glycerophosphate.....	0.1g
Artificial seawater.....	1.0L
Trace elements solution.....	1.0mL

pH 8.0 ± 0.2 at 25°C

**Artificial Seawater:**

**Composition per liter:**

NaCl.....	24.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	4.6g
CaCl <sub>2</sub> .....	1.0g
KCl.....	0.7g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:**

**Composition per liter:**

Disodium EDTA.....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
KBr.....	0.02g
KI.....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
LiCl.....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O.....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to 1.0L of artificial seawater. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

**FUF Medium  
(DSM Medium 318a)**

**Composition per liter:**

KHCO <sub>3</sub> .....	2.0g
NH <sub>4</sub> Cl.....	1.0g
NaCl.....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.08g
Resazurin.....	1.0mg
HEPES solution.....	50.0mL
Furoic acid solution.....	50.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
Cysteine solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL

pH 6.8 ± 0.2 at 25°C

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg

Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Cysteine Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Trace Elements Solution:

**Composition** per liter:

Nitrilotriacetic acid .....	12.8g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.35g
NaCl .....	1.0g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g
MgCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.026g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.025g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 200.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Furoic Acid Solution:

**Composition** per 100.0mL:

2-Furoic acid .....	4.4g
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**Preparation of Furoic Acid Solution:** Add furoic acid to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.0 with NaOH. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### HEPES Solution:

**Composition** per 100.0mL:

HEPES .....	6.2g
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**Preparation of HEPES Solution:** Add HEPES to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except cysteine solution, furoic acid solution,

Na<sub>2</sub>S·9H<sub>2</sub>O solution, and vitamin solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL cysteine solution, 50.0mL furoic acid solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% N<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Methanosarcina thermophila* (*Methanosarcina* sp.) and *Methanosarcina mazei*=*Methanococcus mazei* (*Methanosarcina frisia*).

### Fumarate Medium (DSMZ Medium 195a)

**Composition** per 991.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
pH 7.4 ± 0.2 at 25°C	

#### Solution A:

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.1g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
Na <sub>2</sub> -fumarate .....	2.5g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

#### Solution B (Trace Elements Solution SL-10):

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

# Solution D:

## Composition per 100.0mL:

Resorcinol ..... 1.1g

**Preparation of Solution D:** Add resorcinol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize. Use freshly prepared.

# Solution E (Vitamin Solution):

## Composition per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
p-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.10mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Solution F:

## Composition per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, and 10.0mL solution F. Adjust pH to 7.4 with sodium bicarbonate or sodium carbonate. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth. During growth the culture can be fed with the resorcinol solution.

**Use:** For the cultivation of *Desulfuromusa kysingii*, *Desulfuromusa bakii*, and *Desulfuromusa succinioxidans*.

## *Fundibacter jadensis* Medium (DSMZ Medium 821)

## Composition per liter:

Peptone ..... 2.5g  
Meat extract ..... 1.5g  
Na-Acetate ..... 1.0g  
Artificial seawater, concentrated ..... 190.0mL  
pH 7.2 ± 0.2 at 25°C

## Artificial Seawater, Concentrated:

NaCl ..... 99.4g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 23.76g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 18.12g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 5.2g  
KCl ..... 2.56g

## Preparation of Artificial Seawater, Concentrated:

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1–7.3. Auto-

clave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except concentrated artificial seawater, to distilled/deionized water and bring volume to 810.0mL. Mix thoroughly. Adjust pH to 7.1–7.3. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C. Add 190.0mL concentrated artificial seawater. Mix thoroughly. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation of *Alcanivorax jadensis*.

## Furoate Agar

## Composition per liter:

Agar ..... 20.0g  
2-Furoic acid ..... 2.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g  
NH<sub>4</sub>Cl ..... 1.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus megaterium* and *Pseudomonas* species.

## *Fusibacter paucivorans* Medium (DSMZ Medium 853)

## Composition per 1068mL:

NaCl ..... 30.0g  
Na-Thiosulfate·5H<sub>2</sub>O ..... 3.16g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.0g  
Yeast extract ..... 1.0g  
Trypticase ..... 1.0g  
NH<sub>4</sub>Cl ..... 1.0g  
KCl ..... 1.0g  
Na-acetate·3H<sub>2</sub>O ..... 0.5g  
Cysteine-HCl·H<sub>2</sub>O ..... 0.5g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.3g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
Resazurin ..... 0.5mg  
NaHCO<sub>3</sub> solution ..... 40.0mL  
Glucose solution ..... 18.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL  
Trace elements solution ..... 10.0mL  
pH 7.3 ± 0.2 at 25°C

## Trace Elements Solution:

## Composition per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitrilotriacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O ..... 0.01g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg



**Preparation of Trace Elements Solution:** Add nitrotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 7.0 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### **NaHCO<sub>3</sub> Solution:**

**Composition per 100.0mL:**

NaHCO<sub>3</sub> ..... 10.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 25°C. Must be prepared freshly.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure—121°C. Cool to room temperature.

#### **Glucose Solution:**

**Composition per 50mL:**

Glucose ..... 10.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except glucose solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 25°C. Aseptically and anaerobically add 18.0mL sterile glucose solution, 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 40.0mL sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 7.3. Aseptically and anaerobically distribute into tubes or bottles.

**Use:** For the cultivation of *Fusibacter paucivorans*.

### ***Fusobacterium Medium***

**Composition per liter:**

Agar ..... 15.0g  
Pancreatic digest of casein ..... 15.0g  
Glucose ..... 5.0g  
NaCl ..... 5.0g  
Yeast extract ..... 5.0g  
L-Cysteine ..... 0.75g  
Crystal Violet ..... 0.01g  
Bovine serum ..... 50.0mL  
Streptomycin solution ..... 10.0mL

pH 7.2 ± 0.2 at 25°C

#### **Streptomycin Solution:**

**Composition per 10.0mL:**

Streptomycin ..... 0.01g

**Preparation of Streptomycin Solution:** Add streptomycin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except bovine serum and streptomycin solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 45°–50°C. Aseptically add 50.0mL of sterile bovine serum and 10.0mL of sterile strep-

tomycin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Fusobacterium* species.

### ***Fusobacterium necrophorum Medium***

**Composition per 500.0mL:**

Pancreatic digest of casein ..... 16.0g  
Agar ..... 8.3g  
Biosate ..... 4.0g  
MgSO<sub>4</sub> ..... 2.5g  
Na<sub>2</sub>HPO<sub>4</sub> ..... 2.5g  
Thiotone ..... 2.0g  
Glucose ..... 0.5g  
Egg yolk emulsion, 50% ..... 45.0mL  
Crystal Violet solution ..... 25.0mL  
Phenylethyl alcohol solution ..... 1.35mL

pH 7.3 ± 0.2 at 25°C

#### **Egg Yolk Emulsion, 50%:**

**Composition per 100.0mL:**

Chicken egg yolks ..... 11  
Whole chicken egg ..... 1  
NaCl (0.9% solution) ..... 50.0mL

**Preparation of Egg Yolk Emulsion, 50%:** Soak eggs with 1:100 dilution of saturated mercuric chloride solution for 1 min. Crack 11 eggs, separating yolks from whites. Mix egg yolks with 1 chicken egg. Beat to form emulsion. Combine 50.0mL of egg yolk emulsion and 50.0mL of 0.9% NaCl solution. Mix thoroughly.

#### **Crystal Violet Solution:**

**Composition per 25.0mL:**

Crystal Violet ..... 0.0115g

**Preparation of Crystal Violet Solution:** Aseptically add Crystal Violet to sterile distilled/deionized water and bring volume to 25.0mL. Mix thoroughly.

#### **Phenylethyl Alcohol Solution:**

**Composition per 100.0mL:**

Phenylethyl alcohol ..... 0.27g

#### **Preparation of Phenylethyl Alcohol Solution:**

Aseptically add phenylethyl alcohol to sterile distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components—except egg yolk emulsion, 50%, Crystal Violet solution, and phenylethyl alcohol solution—to distilled/deionized water and bring volume to 428.65mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 45°–50°C. Aseptically add 45.0mL of sterile egg yolk emulsion, 50%, 25.0mL of Crystal Violet solution, and 1.35mL of sterile phenylethyl alcohol solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Fusobacterium necrophorum*.

### **FWM Medium**

**Composition per 1013.0mL:**

Solution A ..... 940.0mL  
Solution E (NaHCO<sub>3</sub> solution) ..... 50.0mL  
Solution F (Substrate solution) ..... 10.0mL  
Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O solution) ..... 10.0mL  
Solution B (Trace elements solution SI-10) ..... 1.0mL  
Solution C (Seven vitamin solution) ..... 1.0mL  
Solution D (Selenite-tungstate solution) ..... 1.0mL

pH 7.2–7.4 at 25°C

**Solution A:****Composition** per 940.0mL:

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Prepare and dispense under 100% N<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C (Seven Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl .....	300.0mg
Nicotinic acid .....	200.0mg
Thiamine-HCl .....	200.0mg
Calcium DL-pantothenate .....	100.0mg
Cyanocobalamin .....	100.0mg
p-Aminobenzoic acid .....	80.0mg
D(+) Biotin .....	20.0mg

**Preparation of Solution C (Seven Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution D (Selenite-Tungstate Solution):****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution D (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution E (NaHCO<sub>3</sub> Solution):****Composition** per 50.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution E (NaHCO<sub>3</sub> Solution):** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F (Substrate Solution):****Composition** per 10.0mL:

Sodium-DL-3-hydroxybutyrate .....	1.5g
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**Preparation of Solution F (Substrate Solution):**

Add sodium-DL-3-hydroxybutyrate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 940.0mL of sterile solution A, aseptically and anaerobically add 1.0mL of sterile solution B, 1.0mL of sterile solution C, 1.0mL of sterile solution D, 50.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Clostridium homopropionicum* and *Desulfococcus biacutus*.

**FWM Medium****Composition** per 1013.0mL:

Solution A .....	940.0mL
Solution E (NaHCO <sub>3</sub> solution) .....	50.0mL
Solution F (Substrate solution) .....	10.0mL
Solution G (Na <sub>2</sub> S·9H <sub>2</sub> O solution) .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution C (Seven vitamin solution) .....	1.0mL
Solution D (Selenite-tungstate solution) .....	1.0mL
pH 7.2–7.4 at 25°C	

**Solution A:****Composition** per 940.0mL:

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Prepare and dispense under 100% N<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C (Seven Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl	300.0mg
Nicotinic acid	200.0mg
Thiamine-HCl	200.0mg
Calcium DL-pantothenate	100.0mg
Cyanocobalamine	100.0mg
<i>p</i> -Aminobenzoic acid	80.0mg
D(+)-Biotin	20.0mg

**Preparation of Solution C (Seven Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution D (Selenite-Tungstate Solution):****Composition** per liter:

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Solution D (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Solution E (NaHCO<sub>3</sub> Solution):****Composition** per 50.0mL:

NaHCO <sub>3</sub>	2.5g
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**Preparation of Solution E (NaHCO<sub>3</sub> Solution):** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution F (Substrate Solution):****Composition** per 10.0mL:

Xylan or xylose	2.0g
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**Preparation of Solution F (Substrate Solution):**

Add 2.0g of xylan or 2.0g of xylose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 940.0mL of sterile solution A, aseptically and anaerobically add 1.0mL of sterile solution B, 1.0mL of sterile solution C, 1.0mL of sterile solution D, 50.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfococcus biacutus*.

**FWN Medium, Modified with Xylan****Composition** per 1020.0mL:

Solution A	950.0mL
Solution C (NaHCO <sub>3</sub> solution)	50.0mL
Solution D (Na <sub>2</sub> S·9H <sub>2</sub> O solution)	10.0mL
Solution B (Wolfe's vitamin solution)	10.0mL

**Solution A:****Composition** per 950.0mL:

Xylan	2.0g
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NaCl	1.0g
KCl	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.4g
NH <sub>4</sub> Cl	0.25g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	0.5mg
Modified Wolfe's mineral solution	10.0mL

**Modified Wolfe's Mineral Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O	0.5g
CaCl <sub>2</sub>	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub>	0.01g
NaWO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.01g

**Preparation of Modified Wolfe's Mineral Solution:**

Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Solution A:** Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Wolfe's Vitamin Solution):****Composition** per liter:

Pyridoxine-HCl	10.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Calcium DL-pantothenate	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Solution B (Wolfe's Vitamin Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution C (NaHCO<sub>3</sub> Solution):****Composition** per 50.0mL:

NaHCO <sub>3</sub>	2.5g
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**Preparation of Solution C (NaHCO<sub>3</sub> Solution):**

Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Solution D (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Au-

toclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Preparation of Medium:** To 950.0mL of sterile solution A, aseptically and anaerobically add 10.0mL of sterile solution B, 50.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks under 100% N<sub>2</sub>.

**Use:** For the cultivation of *Cytophaga xylanolytica*.

### FX A Broth

**Composition per liter:**

Pancreatic digest of casein .....	10.0g
Yeast extract .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### GC Agar with Streptomycin and Chloramphenicol

**Composition per 1030.0mL:**

GC agar base, 2× .....	500.0mL
Hemoglobin solution .....	500.0mL
Supplement solution .....	10.0mL
Streptomycin solution .....	10.0mL
Chloramphenicol solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

**GC Agar Base, 2×:**

**Composition per 500.0mL:**

Agar .....	10.0g
Pancreatic digest of casein .....	7.5g
Peptic digest of animal tissue .....	7.5g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
Cornstarch .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g

**Source:** GC agar base is available as a premixed powder from BD Diagnostic Systems. This base may be replaced by GC medium base available from BD Diagnostic Systems.

**Preparation of GC Agar Base, 2×:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat until boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Hemoglobin Solution:**

**Composition per 500.0mL:**

Bovine hemoglobin .....	10.0g
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**Preparation of Hemoglobin Solution:** Add bovine hemoglobin to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Supplement Solution:**

**Composition per liter:**

Glucose .....	100.0g
L-Cysteine-HCl .....	25.9g
L-Glutamine .....	10.0g
L-Cystine .....	1.1g
Adenine .....	1.0g
Nicotinamide adenine dinucleotide .....	0.25g

Vitamin B <sub>12</sub> .....	0.1g
Thiamine pyrophosphate .....	0.1g
Guanine-HCl .....	0.03g
Fe(NO <sub>3</sub> ) <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
p-Aminobenzoic acid .....	0.013g
Thiamine-HCl .....	3.0mg

**Source:** The supplement solution (IsoVitaleX enrichment) is available from BD Diagnostic Systems. This enrichment may be replaced by supplement VX from BD Diagnostic Systems.

**Preparation of Supplement Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Streptomycin Solution:**

**Composition per 10.0mL:**

Streptomycin .....	0.25g
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**Preparation of Streptomycin Solution:** Add streptomycin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Chloramphenicol Solution:**

**Composition per 10.0mL:**

Chloramphenicol .....	25.0mg
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**Preparation of Chloramphenicol Solution:** Add chloramphenicol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 500.0mL of sterile GC agar base, aseptically add 500.0mL of sterile hemoglobin solution at 45°–50°C. Mix thoroughly. Aseptically add 10.0mL of sterile supplement solution, 10.0mL of sterile ampicillin solution, and 10.0mL of sterile tetracycline solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Azorhizophilus paspali*.

### Gelatin Agar

**Composition per liter:**

Agar .....	15.0g
Gelatin .....	15.0g
Peptone .....	4.0g
Yeast extract .....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of heterotrophic bacteria based upon their utilization of gelatin.

### General Salts Medium for Estuarine Methanogens

**Composition per 410.8mL:**

Agar .....	8.0g
NaCl .....	3.6g
NaHCO <sub>3</sub> .....	2.0g
Complete salts solution .....	200.0mL
Cysteine-sulfide reducing agent .....	16.0mL
Wolfe's mineral solution .....	4.0mL
Vitamin solution .....	4.0mL
Yeast extract–Trypticase solution .....	4.0mL
Sodium acetate (25% solution) .....	2.0mL

Fe(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (0.2% solution) .....	0.4mL
Resazurin (0.1% solution) .....	0.4mL
pH 7.0 ± 0.2 at 25°C	

**Complete Salts Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.9g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.5g
KCl .....	0.67g
NH <sub>4</sub> Cl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.28g
K <sub>2</sub> HPO <sub>4</sub> .....	0.28g

**Preparation of Complete Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Cysteine-Sulfide Reducing Agent:****Composition per 400.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	5.0g
Na <sub>2</sub> S (12.5% solution) .....	40.0mL
NaOH (1N solution) .....	30.0mL

**Preparation of Cysteine-Sulfide Reducing Agent:**

Add distilled/deionized water to a 500.0mL round-bottomed flask. Add freshly prepared NaOH solution. Gently heat and bring to boiling under 100% N<sub>2</sub>. Remove gassing probe. Add L-cysteine-HCl·H<sub>2</sub>O. Add freshly prepared Na<sub>2</sub>S solution. Renew gassing for several minutes. Cap with rubber stoppers. Distribute into 8.0mL/18mm Hungate tubes.

**Yeast Extract-Trypticase Solution:****Composition per 100.0mL:**

Yeast extract .....	20.0g
Pancreatic digest of casein .....	20.0g

**Preparation of Yeast Extract-Trypticase Solution:**

Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriloacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except cysteine-sulfide reducing agent, to distilled/deionized water and bring volume to 410.8mL. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add cysteine-sulfide reducing agent. Continue boiling until resazurin turns colorless, indicating reduction. Distribute anaerobically into culture tubes with aluminum crimp seals. Autoclave for 15 min at 15 psi pressure—121°C.

**Use:** For the cultivation and maintenance of *Methanococcus deltae*, *Methanococcus vannielii*, *Methanococcus vol-*

*tae*, *Methanogenium cariaci*, *Methanogenium marisnigri*, and *Methanogenium olentangyi*.

**Geo Medium****Composition per liter:**

Agar .....	15.0g
CaCO <sub>3</sub> .....	1.0g
Glucose .....	1.0g
Starch, soluble .....	1.0g
Yeast extract .....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure—121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Geodermatophilus obscurus*.

**Geobacter Medium  
(DSMZ Medium 579)****Composition per liter:**

Fe(III) citrate .....	13.7g
NaHCO <sub>3</sub> .....	2.5g
Na-acetate .....	2.5g
NH <sub>4</sub> Cl .....	1.5g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.6g
KCl .....	0.1g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	0.25mg
Vitamin solution .....	10.0mL
Trace elements solution .....	10.0mL

pH 6.7-7.0 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Dissolve ferric citrate in 900.0mL distilled/deionized water by heating and adjust to pH 6.0. Add other components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Geobacter metallireducens* and *Geobacter grbiciae*.

### ***Geobacter* Medium (DSMZ Medium 826)**

**Composition** per 1050mL:

NaHCO <sub>3</sub> .....	2.5g
NH <sub>4</sub> Cl.....	1.5g
Na-acetate.....	0.82g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.6g
KCl.....	0.1g
Resazurin.....	0.5mg
Na-fumarate solution.....	50.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
Selenite-tungstate solution.....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Na-Fumarate Solution:**

**Composition** per 100.0mL:

Na-fumarate.....	16.0g
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**Preparation of Na-Fumarate Solution:** Add Na-fumarate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Selenite-Tungstate Solution**

**Composition** per liter:

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except NaHCO<sub>3</sub> and fumarate solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add the NaHCO<sub>3</sub>. Equilibrate with the 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH of 6.8. Distribute into anaerobe tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically add 0.5mL Na-fumarate solution per 10.0mL medium. The pH should be 6.8.

**Use:** For the cultivation of *Geobacter sulfurreducens*, *Geobacter bremsensis* (*Geobacter* sp.), *Geobacter chapellei*, *Geobacter hydrogenophilus*, and *Geothrix fermentans*.

### ***Geodermatophilus obscurus* Medium**

**Composition** per liter:

Agar.....	20.0g
Malt extract, purified solids.....	15.0g
Starch, soluble.....	10.0g
Sucrose.....	10.0g
Yeast extract.....	5.0g
CaCO <sub>3</sub> .....	2.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Geodermatophilus obscurus*.

### **George's Medium, Modified**

**Composition** per liter:

Agar.....	15.0g
Peptone.....	1.0g
KNO <sub>3</sub> .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.02g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.02g
Ferric citrate.....	0.035mg

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min

at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of algae.

### GFY Agar

#### Composition per liter:

Agar .....	15.0g
Glucose .....	5.0g
Fructose .....	5.0g
Yeast extract .....	5.0g
CaCO <sub>3</sub> .....	3.0g
Vitamin B <sub>12</sub> .....	2.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flexibacter* species.

### Gisa Agar

#### Composition per liter:

Agar .....	18.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	5.0g
Calcium glucoisaccharinate .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Inositol .....	2.0mg
KI .....	1.0mg
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.4mg
Thiamine-HCl .....	0.4mg
Pyroxidine-HCl .....	0.4mg
Niacin .....	0.4mg
Calcium pantothenate .....	0.4mg
<i>p</i> -Aminobenzoic acid .....	0.2mg
Riboflavin .....	0.2mg
FeCl <sub>3</sub> .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.2mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04mg
Folic acid .....	2.0μg
Biotin .....	2.0μg

pH 5.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Ancylobacter aquaticus*, *Pseudomonas* species, *Xanthobacter autotrophicus*, and *Xanthobacter* species.

### Gliding Medium

#### Composition per liter:

Pancreatic digest of casein .....	0.5g
Yeast extract .....	0.5g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of the gliding bacteria *Cytophaga* species and *Flexibacter columnaris*.

### *Gluconacetobacter johannae* *Gluconacetobacter azotocaptans* Medium (DSMZ Medium 920)

#### Composition per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	4.81g
MES buffer .....	4.4g
Yeast extract .....	2.7g
Glucose .....	2.7g
Mannitol .....	1.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.65g

pH 6.7 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Gluconacetobacter azotocaptans* and *Gluconacetobacter johannae*.

### Gluconate Peptone Broth

#### Composition per liter:

Potassium gluconate .....	40.0g
Casein peptone .....	1.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Yeast extract .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of Gram-negative bacteria based on their ability to oxidize gluconate to 2-ketogluconate. For the differentiation of fluorescent *Pseudomonas* species. After inoculation with bacteria and 48 hr of growth in this medium, Benedict's reagent is added. Bacteria that produce the reducing sugar 2-ketogluconate turn the reagent yellow-orange to orange-red.

### Glucose Agar, 9K

#### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.0125g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01mg
Agar solution .....	500.0mL
Glucose solution .....	100.0mL

pH 4.5 ± 0.2 at 25°C

#### Agar Solution:

##### Composition per 500.0mL:

Agar .....	15.0g
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**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C.

#### Glucose Solution:

##### Composition per 100.0mL:

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except agar solution and glucose solution, to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 4.5 with  $\text{H}_2\text{SO}_4$ . Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C. Aseptically add 500.0mL of sterile agar solution and 100.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thiobacillus acidophilus*.

#### Glucose Agar with 25%Glucose

**Composition** per liter:

Glucose .....	250.0g
Agar .....	25.0g
Polypeptone .....	5.0g
Malt extract .....	3.0g
Yeast extract .....	3.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of osmophilic yeasts and bacteria.

#### Glucose Broth

**Composition** per 800.0mL:

Agar .....	10.0g
Beef extract .....	10.0g
Peptone.....	10.0g
NaCl .....	5.0g
Glucose solution .....	200.0mL

pH 7.0 ± 0.2 at 25°C

**Glucose Solution:**

**Composition** per 200.0mL:

Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically add 200.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Pseudomonas* species.

#### Glucose Broth, 9K

**Composition** per liter:

Glucose .....	10.0g
$(\text{NH}_4)_2\text{SO}_4$ .....	3.0g
$\text{KH}_2\text{PO}_4$ .....	0.5g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5g
KCl .....	0.1g
$\text{Ca}(\text{NO}_3)_2$ .....	0.0125g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.01mg
Glucose solution .....	100.0mL

pH 3.5 ± 0.2 at 25°C

**Glucose Solution:**

**Composition** per 100.0mL:

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Adjust pH to 3.5 with  $\text{H}_2\text{SO}_4$ . Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus acidophilus*.

#### Glucose Nitrogen-Free Salt Agar

**Composition** per liter:

Agar .....	15.0g
$\text{CaCO}_3$ .....	1.0g
$\text{K}_2\text{HPO}_4$ .....	1.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.2g
NaCl .....	0.2g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	5.0mg
Glucose solution .....	100.0mL

pH 7.0 ± 0.2 at 25°C

**Glucose Solution:**

**Composition** per 100.0mL:

Glucose.....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Azotobacter* species.

#### Glucose Nitrogen-Free Salt Solution

**Composition** per liter:

$\text{CaCO}_3$ .....	1.0g
$\text{K}_2\text{HPO}_4$ .....	1.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.2g
NaCl .....	0.2g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	5.0mg
Glucose solution .....	100.0mL

pH 7.0 ± 0.2 at 25°C

**Glucose Solution:**

**Composition** per 100.0mL:

Glucose.....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Azotobacter* species.



**Glucose Peptone Agar****Composition** per liter:

Peptone.....	20.0g
Agar .....	15.0g
Glucose .....	10.0g
NaCl .....	5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Agrobacterium* species.

**Glucose Yeast Chalk Agar****Composition** per liter:

Chalk.....	40.0g
Agar .....	15.0g
Glucose .....	5.0g
Yeast extract.....	5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas* species.

**Glucose Yeast Extract Agar****Composition** per liter:

CaCO <sub>3</sub> .....	20.0g
Glucose .....	20.0g
Agar .....	17.0g
Yeast extract.....	10.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Agrobacterium* species, *Clostridium* species, *Erwinia* species, *Pseudomonas* species, and *Xanthomonas campestris*.

**Glucose Yeast Extract Iron Agar  
(LMG Medium 153)****Composition** per liter:

Agar .....	15.0g
Glucose .....	10.0g
Yeast extract.....	5.0g
Iron solution.....	20.0mL

pH 7.1 ± 0.2 at 25°C

**Iron Solution:****Composition** per 100.0mL:

FeCl <sub>3</sub> .....	0.03g
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**Preparation of Iron Solution:** Add FeCl<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except iron solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 20.0mL sterile iron solution. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Pseudomonas denitrificans*.

**Glucose Yeast Extract Medium  
(ATCC Medium 985)****Composition** per liter:

Agar.....	15.0g
Yeast extract .....	3.0g
Glucose.....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Acinetobacter tartarogenes*, *Agrobacterium viscosum*, and *Pseudomonas* species.

**Glucose Yeast Medium  
with Calcium Carbonate****Composition** per liter:

Agar.....	15.0g
CaCO <sub>3</sub> .....	7.5g
Peptone .....	5.0g
Yeast extract .....	5.0g
Glucose.....	3.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Adjust pH to 6.3. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Erwinia herbicola* and *Bacillus* species.

**Glucose Yeast Peptone Medium****Composition** per liter:

Glucose.....	10.0g
Peptone .....	5.0g
Yeast extract .....	5.0g

pH 5.0 ± 1.0 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Enterobacter cloacae*.

**Glutamate Medium****Composition** per liter:

Solution A .....	500.0mL
Solution B.....	250.0mL
Solution C.....	250.0mL

**Solution A:****Composition** per 500.0mL:

Mannitol .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.22g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B:****Composition** per 250.0mL:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.08g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.05g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution C:

**Composition per 250.0mL:**

Sodium glutamate .....	1.1g
Calcium pantothenate .....	0.5mg
Thiamine-HCl .....	0.1mg
Biotin .....	0.5µg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 500.0mL of cooled, sterile solution A, 250.0mL of cooled, sterile solution B, and 250.0mL of sterile solution C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation of *Rhizobium* species.

#### Glutamate Medium (ATCC Medium 820)

**Composition per liter:**

Agar .....	15.0g
Sodium glutamate .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
KCl .....	0.1g
Glucose solution .....	100.0mL

pH 6.5 ± 0.2 at 25°C

#### Glucose Solution:

**Composition per 100.0mL:**

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

#### Glycerol Agar

**Composition per 1070.0mL:**

Agar .....	15.0g
Peptone .....	5.0g
Beef extract .....	3.0g
Soil extract .....	1.0L
Glycerol .....	70.0mL

pH 7.0 ± 0.2 at 25°C

#### Soil Extract:

**Composition per liter:**

Soil, airdried .....	1.0Kg
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**Preparation of Soil Extract:** Sift soil through a #9 mesh screen. Add to 2.4L of tap water. Mix thoroughly. Autoclave for 60 min at 15 psi pressure–121°C. Cool to 25°C. Filter through Whatman #1 filter paper. Bring volume to 1.0L with tap water.

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Autoclave for

15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of *Nocardia* species and *Rhodococcus* species.

#### Glycerol Agar

**Composition per liter:**

Beef heart, solids from infusion .....	250.0g
Glycerol .....	60.0g
Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Tryptose .....	5.0g
Beef extract .....	3.0g
NaCl .....	2.5g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus subtilis*, *Enterococcus faecalis*, *Erwinia chrysanthemi*, *Gordona rubropertinctus*, *Mycobacterium* species, *Nocardia brevicatena*, *Rhodococcus equi*, and *Rhodococcus rhodochrous*.

#### Glycerol Arginine Agar

**Composition per liter:**

Agar .....	15.0g
Glycerol .....	12.5g
Arginine .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation and cultivation of streptomycetes.

#### Glycerol Chalk Agar

**Composition per liter:**

NaCl .....	30.0g
Agar .....	15.0g
Glycerol .....	10.0g
CaCO <sub>3</sub> .....	5.0g
Peptone .....	5.0g
Yeast extract .....	3.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. Swirl flask while dispensing medium to keep CaCO<sub>3</sub> in suspension.

**Use:** For the cultivation of *Photobacterium* species and *Lucibacterium* species.

#### GÖ1 Medium

**Composition per liter:**

Sucrose.....	17.1g
Sodium acetate.....	10.0g
NaCl.....	2.25g
Pancreatic digest of casein.....	2.0g
Yeast extract.....	2.0g
NaHCO <sub>3</sub> .....	0.85g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
NH <sub>4</sub> Cl.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0mg
Resazurin.....	1.0mg
NaHCO <sub>3</sub> solution.....	40.0mL
Vitamin solution.....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 6.5–6.8 at 25°C	

**NaHCO<sub>3</sub> Solution:****Composition per 50.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	10.0mg
DL-Calcium pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**L-Cysteine-HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O.....	0.3g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg

H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:**

Add components, except NaHCO<sub>3</sub> solution, vitamin solution, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Sparge under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 40.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile vitamin solution, 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Methanosarcina mazei*.

**Gorbenko Medium****Composition per liter:**

Agar.....	16.2g
Peptone.....	0.9g
NaCl.....	0.9g
Yeast extract.....	0.36g
Beef extract.....	0.18g
Lake water.....	1000.0mL

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Gordonia rubropertinctus*, *Gordonia terrae*, and heterotrophic marine bacteria.

**Gorham's Medium for Algae****Composition per liter:**

NaNO <sub>3</sub> .....	0.496g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.075g
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O.....	0.058g
K <sub>2</sub> HPO <sub>4</sub> .....	0.039g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.036g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid.....	6.0mg
EDTA.....	6.0mg
Ferric citrate.....	6.0mg

pH 7.5 ± 0.5 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Anabaena flos-aquae*, *Selenastrum capricornutum* and *Microcystis aeruginosa*.

**GPVA Medium****Composition per liter:**

Agar.....	15.0g
Yeast extract.....	10.0g

ACES buffer (2-[(2-Amino-2-oxoethyl)-amino]-ethane sulfonic acid) .....	10.0g
Glycine.....	3.0g
Charcoal, activated.....	2.0g
$\alpha$ -Ketoglutarate.....	1.0g
$\text{Fe}_4(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$ .....	0.25g
Antibiotic inhibitor solution.....	10.0mL
pH $6.9 \pm 0.2$ at $25^\circ\text{C}$	

**Antibiotic Inhibitor Solution:****Composition per 10.0mL:**

Anisomycin.....	0.08g
Vancomycin.....	5.0mg
Polymyxin B.....	100,000U

**Preparation of Antibiotic Inhibitor Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except antibiotic inhibitor solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure— $121^\circ\text{C}$ . Cool to  $45^\circ\text{--}50^\circ\text{C}$ . Adjust pH to 6.9. Aseptically add 10.0mL of sterile antibiotic inhibitor solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Legionella* species from environmental waters.

**GPY Salts Medium  
(Glucose Peptone Yeast  
Extract Salts Medium)**

**Composition per liter:**

Glucose.....	1.0g
Peptone.....	0.5g
Yeast extract.....	0.1g
Modified Hutner's mineral base.....	20.0mL

**Hutner's Mineral Base:****Composition per liter:**

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	29.7g
Nitrilotriacetic acid.....	10.0g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	3.34g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	99.0mg
$(\text{NH}_4)_2\text{MoO}_4$ .....	9.25mg
"Metals 44".....	50.0mL

**Preparation of Hutner's Mineral Base:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with  $\text{H}_2\text{SO}_4$  or KOH. Add distilled/deionized water to 1.0L. Store at  $5^\circ\text{C}$ .

**"Metals 44":****Composition per 100.0mL:**

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	1.1g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5g
EDTA.....	0.25g
$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.154g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .....	0.04g
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ .....	0.025g
$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ .....	0.018g

**Preparation of "Metals 44":** Add a few drops of  $\text{H}_2\text{SO}_4$  to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure— $121^\circ\text{C}$ .

**Use:** For the cultivation and maintenance of *Prostheco-bacter fusiformis*.

**GYM + Seawater  
(DSMZ Medium 871)**

**Composition per liter:**

Sea salts.....	32.0g
Agar.....	15.0g
Malt extract.....	10.0g
Glucose.....	4.0g
Yeast extract.....	4.0g
$\text{CaCO}_3$ .....	2.0g
pH $7.0\text{--}7.4$ at $25^\circ\text{C}$	

**Preparation of Medium:** Add sea salts to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add remaining components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure— $121^\circ\text{C}$ .

**Use:** For the cultivation of *Nocardiosis aegyptia* (*Nocardiosis* sp.) and *Nocardiosis halotolerans* (*Nocardiosis* sp.).

**GYM Starch Agar  
(DSMZ Medium 214)**

**Composition per liter:**

Starch.....	20.0g
Agar.....	12.0g
Malt extract.....	10.0g
Glucose.....	4.0g
Yeast extract.....	4.0g
$\text{CaCO}_3$ .....	2.0g
pH $7.2 \pm 0.2$ at $25^\circ\text{C}$	

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure— $121^\circ\text{C}$ . Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Amycolatopsis orientalis* subsp. *Orientalis*, *Nocardia* spp., *Mycobacterium* spp., *Pseudonocardia* spp., *Saccharothrix* spp., *Kineosporia aurantiaca*, *Kitasatospora setae*, *Oerskovia turbata* (*Cellulomonas turbata*), *Cellulosimicrobium cellulans*, and *Thermoactinomyces dichotomicus*.

**GYM Streptomyces Agar  
(DSMZ Medium 65)**

**Composition per liter:**

Agar.....	12.0g
Malt extract.....	10.0g
Glucose.....	4.0g
Yeast extract.....	4.0g
$\text{CaCO}_3$ .....	2.0g
pH $7.2 \pm 0.2$ at $25^\circ\text{C}$	

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure— $121^\circ\text{C}$ . Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Streptomyces* spp.

**Hagedorn and Holt Selective Medium****Composition per liter:**

NaCl .....	20.0g
Agar .....	15.0g
Yeast extract .....	2.0g
Pancreatic digest of casein .....	1.7g
Agar .....	1.5g
NaCl .....	0.5g
Papaic digest of soybean meal .....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
Glucose .....	0.25g
Cycloheximide .....	0.1g
Methyl Red .....	0.15mg

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation of *Arthrobacter* spp. in soil.

**Haloalkaliphilic Agar****Composition per liter:**

Solution A .....	900.0mL
Solution B .....	100.0mL

pH 8.5–9.5 at 25°C

**Solution A:****Composition per 900.0mL:**

NaCl .....	200.0g
Agar .....	25.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Sodium citrate .....	3.0g
KCl .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.25mg

**Preparation of Solution A:** Add components, except NaCl, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Add 200.0g of NaCl. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Solution B:****Composition per 100.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
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**Preparation of Solution B:** Dissolve 5.0g of Na<sub>2</sub>CO<sub>3</sub> in distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically mix 900.0mL of solution A and 100.0mL of solution B. Mix thoroughly. Aseptically adjust pH to 8.5–9.5. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Natronobacterium gregoryi*, *Natronobacterium magadii*, *Natronobacterium pharaonis*, and *Natronococcus occultus*.

**Haloanaerobacter chitinovorans Medium****Composition per 1001.0mL:**

NaCl .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	7.0g
Chitin .....	5.0g
KCl .....	3.8g

Na <sub>2</sub> CO <sub>3</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.4g
Resazurin .....	0.001g
Na <sub>2</sub> CO <sub>3</sub> .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-6 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition per 20.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	3.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**L-Cysteine·HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.5g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, substrate solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and L-cysteine·HCl·H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile substrate solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Haloanaerobacter chitinovorans*.

**Haloanaerobium alcaliphilum Medium  
(DSMZ Medium 807)****Composition per liter:**

NaCl .....	100.0g
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MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	17.0g
Trypticase™ .....	10.0g
NaHCO <sub>3</sub> .....	4.1g
Cysteine-HCl·H <sub>2</sub> O .....	0.5g
Resazurin .....	1.0mg
Solution A .....	50.0mL
Solution B .....	50.0mL
Yeast extract solution .....	50.0mL
Glucose solution .....	20.0mL
Trace elements solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.0 ± 0.1 at 25°C

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution: Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Glucose Solution: Composition per 20mL:

Glucose .....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

#### Yeast Extract Solution: Composition per 50mL:

Yeast extract .....	10.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Solution A: Composition per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
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**Preparation of Solution A:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution B: Composition per liter:

NaCl .....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.6g
NH <sub>4</sub> Cl .....	2.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.28g
K <sub>2</sub> HPO <sub>4</sub> .....	0.28g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution: Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub>, Na<sub>2</sub>S·9H<sub>2</sub>O solution, cysteine-HCl·H<sub>2</sub>O, yeast extract solution, and glucose solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add 4.1g NaHCO<sub>3</sub> and 0.5g cysteine-HCl·H<sub>2</sub>O. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL sterile glucose solution, 50.0mL sterile yeast extract solution, and 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Haloanaerobium alcaliphilum*.

#### Haloanaerobium congolense Medium (DSMZ Medium 933)

##### Composition per 1080mL:

NaCl .....	100.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.0g
Trypticase .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
KCl .....	1.0g
Na-acetate .....	0.5g
Cysteine .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	0.01g
Glucose solution .....	20.0mL
Thiosulfate solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
NaHCO <sub>3</sub> solution .....	20.0mL
Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Solution: Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.2g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**NaHCO<sub>3</sub> Solution:****Composition** per 50.0mL:NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Thiosulfate Solution:****Composition** per 20mL:Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 5.0g

**Preparation of Thiosulfate Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Glucose Solution:****Composition** per 20.0mL:

Glucose ..... 3.5g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, glucose solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and thiosulfate solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 7.0. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically add per 10.0mL medium, 0.2mL NaHCO<sub>3</sub> solution, 0.2mL glucose solution, 0.2mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 0.2mL thiosulfate solution. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Thermococcus waiotapuensis*.

***Haloanaerobium lacusroseus* Medium  
(DSMZ Medium 764)**

**Composition** per liter:

NaCl ..... 200.0g  
KCl ..... 4.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 2.0g  
Yeast extract ..... 1.0g  
NH<sub>4</sub>Cl ..... 1.0g  
Na-acetate ..... 1.0g  
Trypticase™ ..... 0.5g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.3g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.2g  
Resazurin ..... 0.001g  
Trace elements SL-6 ..... 1.0mL  
Glucose solution ..... 100.0mL  
NaHCO<sub>3</sub> solution ..... 50.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL  
Dithionite solution ..... 5.0mL

pH 7.0 ± 0.2 at 25°C

**Glucose Solution:****Composition** per 100mL:

Glucose ..... 17.4g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-6:****Composition** per liter:

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.5g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.03g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02g  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.2g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Neutralize to pH 7.0 with sterile HCl.

**NaHCO<sub>3</sub> Solution:****Composition** per 100.0mL:NaHCO<sub>3</sub> ..... 10.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Dithionite Solution:****Composition** per 10mL:

Na-dithionite ..... 2.0mg

**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except glucose solution, NaHCO<sub>3</sub> solution, dithionite solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 835.0mL. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Cool while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into Hungate tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically inject glucose solution (0.25mL/10mL medium), dithionite solution (0.05mL/10mL medium), NaHCO<sub>3</sub> solution (0.5mL/10mL medium), and Na<sub>2</sub>S·9H<sub>2</sub>O solution (0.1mL/10mL medium). Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Haloanaerobium lacusrosei* (*Haloanaerobium lacusroseus*).

***Haloanaerobium praevalens* Medium**

**Composition** per liter:

NaCl ..... 130.0g  
Agar ..... 20.0g  
Yeast extract ..... 2.0g  
Pancreatic digest of casein ..... 2.0g

NH <sub>4</sub> Cl.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.35g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.23g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0mg
NaHCO <sub>3</sub> solution.....	20.0mL
L-Cysteine-sulfide reducing agent.....	20.0mL
Wolfe's vitamin solution.....	10.0mL
Methanol.....	10.0mL
Resazurin (0.025% solution).....	4.0mL
Trace elements SL-6.....	3.0mL
pH 6.8 ± 0.2 at 25°C	

### NaHCO<sub>3</sub> Solution:

**Composition** per 20.0mL:

NaHCO <sub>3</sub> .....	850.0mg
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Gas with 100% CO<sub>2</sub> for 20 min.

### L-Cysteine-Sulfide Reducing Agent:

**Composition** per 20.0mL:

L-Cysteine-HCl·H <sub>2</sub> O.....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g

**Preparation of L-Cysteine-Sulfide Reducing Agent:** Add L-cysteine-HCl·H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. In a separate tube, add Na<sub>2</sub>S·9H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. Gas both solutions with 100% N<sub>2</sub> and cap tubes. Autoclave both solutions for 15 min at 15 psi pressure–121°C using fast exhaust. Cool to 50°C. Aseptically combine the two solutions under 100% N<sub>2</sub>.

### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
Calcium pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, L-cysteine-sulfide reducing agent, Wolfe's vitamin solution, and methanol, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add the

sterile NaHCO<sub>3</sub> solution, the sterile L-cysteine-sulfide reducing agent, the sterile Wolfe's vitamin solution, and filter-sterilized methanol. Mix thoroughly. Adjust pH to 6.8. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Haloanaerobium praevalens*.

### Haloanaerobium salsugo Medium

**Composition** per liter:

NaCl.....	90.0g
Purified agar (if necessary).....	20.0g
Casamino acids.....	5.0g
Yeast extract.....	5.0g
Dipotassium PIPES (piperazine- <i>N,N'</i> -bis[2-ethanesulfonic acid]) buffer.....	1.5g
Resazurin.....	1.0mg
Glucose solution.....	50.0mL
L-Cysteine-sulfide reducing solution.....	20.0mL
Mineral solution.....	20.0mL
Wolfe's vitamin solution.....	10.0mL
Modified Wolfe's mineral solution.....	5.0mL
pH 6.0–7.0 at 25°C	

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except glucose solution and L-cysteine-sulfide reducing solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Adjust pH to 6.0–7.0. Add 20.0mL of L-cysteine-sulfide reducing solution. Mix thoroughly. Anaerobically distribute 9.5mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.5mL of sterile glucose solution to each tube. Mix thoroughly.

### Glucose Solution:

**Composition** per 50.0mL:

D-Glucose.....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Mineral Solution:

**Composition** per liter:

NH <sub>4</sub> Cl.....	50.0g
NaCl.....	40.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0g
KCl.....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0g

### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Calcium DL-pantothenate.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.



**Modified Wolfe's Mineral Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.01g
NaWO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Modified Wolfe's Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**L-Cysteine-Sulfide Reducing Solution:****Composition** per 200.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	5.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	5.0g
NaOH .....	1.25g

**Preparation of L-Cysteine-Sulfide Reducing Solution:** Add NaOH to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add L-cysteine-HCl·H<sub>2</sub>O and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Anaerobically distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloanaerobium salsaugo*.

***Haloarcula japonica* Medium****Composition** per liter:

NaCl .....	200.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Trisodium citrate·2H <sub>2</sub> O .....	3.0g
KCl .....	2.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.36mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloarcula japonica*.

***Haloarcula marismortui* Medium****Composition** per liter:

NaCl .....	208.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	46.6g
Yeast extract .....	10.0g
CaCl <sub>2</sub> .....	0.5g
MnCl <sub>2</sub> .....	0.125g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloarcula marismortui*.

***Haloarcula vallismortis*****Synthetic Medium****Composition** per 1029.0mL:

Basal salts solution .....	1.0L
Glucose solution .....	20.0mL
NH <sub>4</sub> Cl solution .....	5.0mL
FeSO <sub>4</sub> ·6H <sub>2</sub> O solution .....	2.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	2.0mL

pH 7.5 ± 0.2 at 25°C

**Basal Salts Solution:****Composition** per liter:

NaCl .....	200.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	36.0g
Tris[hydroxymethyl]aminomethane .....	6.0g
KCl .....	4.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g

**Preparation of Basal Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with HCl. Autoclave for 15 min at 15 psi pressure–121°C.

**FeSO<sub>4</sub>·6H<sub>2</sub>O Solution:****Composition** per 100.0mL:

FeSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.2g
HCl (1.0M solution) .....	100.0mL

**Preparation of FeSO<sub>4</sub>·6H<sub>2</sub>O Solution:** Combine components. Mix thoroughly. Filter sterilize.

**K<sub>2</sub>HPO<sub>4</sub> Solution:****Composition** per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	5.0g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Combine components. Mix thoroughly. Filter sterilize.

**NH<sub>4</sub>Cl Solution:****Composition** per 100.0mL:

NH <sub>4</sub> Cl .....	20.0g
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**Preparation of NH<sub>4</sub>Cl Solution:** Combine components. Mix thoroughly. Filter sterilize.

**Glucose Solution:****Composition** per 100.0mL:

D-Glucose .....	25.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Glucose Solution:** Aseptically combine 1.0L of sterile basal salts solution with 20.0mL of sterile glucose solution, 5.0mL of sterile NH<sub>4</sub>Cl solution, 2.0mL of sterile K<sub>2</sub>HPO<sub>4</sub> solution, and 2.0mL of sterile FeSO<sub>4</sub>·6H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Haloarcula vallismortis*.

***Halobacillus* Medium****(DSMZ Medium 755)****Composition** per liter:

NaCl .....	100.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
Peptone, casein digest .....	5.0g
Yeast extract .....	3.0g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halobacillus trueperi* and *Halobacillus litoralis*.

#### Halobacteria Medium

##### Composition per liter:

NaCl .....	220.0g
Agar .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0g
Casein hydrolysate .....	5.0g
KCl .....	5.0g
Disodium citrate .....	3.0g
KNO <sub>3</sub> .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g

pH 7.2–7.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 7.2–7.4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and enumeration of halobacteria.

#### Halobacteriaceae Medium 1

##### Composition per liter:

Salt, crude solar .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
KCl .....	5.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the axenic cultivation of members of the Halobacteriaceae.

#### Halobacteriaceae Medium 2

##### Composition per liter:

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Trisodium citrate .....	3.0g
KCl .....	2.0g
FeCl <sub>2</sub> .....	2.3mg

pH 7.5–7.8 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 7.5–7.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the axenic cultivation of halobacteria and halococci.

#### Halobacteriaceae Medium 3

##### Composition per liter:

NaCl .....	240.0g
L-Glutamine .....	15.0g
KCl .....	5.0g
K <sub>2</sub> SO <sub>4</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0g
MgSO <sub>4</sub> , anhydrous .....	5.0g

NH <sub>4</sub> Cl .....	5.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
L-Arginine .....	0.5g
L-Isoleucine .....	0.25g
L-Leucine .....	0.25g
L-Lysine .....	0.25g
L-Proline .....	0.25g
L-Valine .....	0.25g
Cytidylic acid .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
L-Methionine .....	0.1g
L-Tyrosine .....	0.1g
L-Phenylalanine .....	0.05g
FeCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of some halobacteria and halococci.

#### Halobacteriaceae Medium 4

##### Composition per liter:

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
NH <sub>4</sub> Cl .....	5.0g
L-Glutamic acid .....	1.3g
DL-Valine .....	1.0g
Glycerol .....	1.0g
L-Lysine .....	0.85g
L-Leucine .....	0.8g
DL-Serine .....	0.61g
DL-Threonine .....	0.5g
DL-Isoleucine .....	0.44g
DL-Alanine .....	0.43g
L-Arginine .....	0.4g
DL-Methionine .....	0.37g
DL-Phenylalanine .....	0.26g
L-Tyrosine .....	0.2g
Adenylic acid .....	0.1g
KNO <sub>3</sub> .....	0.1g
Uridylic acid .....	0.1g
Glycine .....	0.06g
KH <sub>2</sub> PO <sub>4</sub> .....	0.05g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
L-Cysteine .....	0.05g
L-Proline .....	0.05g
Sodium citrate .....	0.05g
FeCl <sub>2</sub> .....	2.3mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.7mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.44mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.3mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.05mg

pH 6.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 6.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of members of the Halobacteriaceae.

***Halobacterium Agar*****Composition per liter:**

NaCl .....	250.0g
Agar .....	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Trisodium citrate .....	3.0g
KCl .....	2.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.2mg

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Haloarcula* species, *Halobacterium* species, *Halococcus morrhuae*, *Haloferax mediterranei*, and *Haloferax volcanii*.

***Halobacterium denitrificans* Medium****Composition per liter:**

NaCl .....	176.0g
Agar .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0g
HEPES ( <i>N</i> -2-Hydroxyethylpiperazine- <i>N'</i> - 2-ethanesulfonic acid) buffer .....	11.9g
Yeast extract .....	5.0g
Hy-Case SF (Humko-Sheffield) .....	2.0g
KCl .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

pH 6.7 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.7. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the aerobic cultivation and maintenance of *Haloferax* (*Halobacterium*) *denitrificans*.

***Halobacterium denitrificans* Medium****Composition per liter:**

NaCl .....	176.0g
Agar .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0g
HEPES ( <i>N</i> -2-Hydroxyethylpiperazine- <i>N'</i> - 2-ethanesulfonic acid) buffer .....	11.9g
KNO <sub>3</sub> .....	5.0g
Yeast extract .....	5.0g
Hy-Case SF (Humko-Sheffield) .....	2.0g
KCl .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

pH 6.7 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.7. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the anerobic cultivation and maintenance of *Haloferax* (*Halobacterium*) *denitrificans*.

***Halobacterium halobium* Defined Medium****Composition per liter:**

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
L-Glutamic acid .....	1.3g
KCl .....	1.0g
Glycerol .....	1.0g
KCl .....	1.0g
L-Valine .....	1.0g
L-Lysine .....	0.85g
L-Leucine .....	0.8g
CaCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.71g
L-Serine .....	0.6g
Sodium citrate·2H <sub>2</sub> O .....	0.5g
L-Proline .....	0.5g
L-Threonine .....	0.5g
L-Alanine .....	0.43g
L-Arginine .....	0.4g
L-Methionine .....	0.37g
L-Phenylalanine .....	0.26g
L-Tyrosine .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.15g
KNO <sub>3</sub> .....	0.1g
KNO <sub>3</sub> .....	0.1g
Glycine .....	0.06g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.05g
L-Isoleucine .....	0.044g
FeCl <sub>2</sub> ·5H <sub>2</sub> O .....	2.3mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.44mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.005mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halobacterium halobium*.

***Halobacterium halobium*/  
*Halobacterium salinarium* Medium****Composition per liter:**

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	3.0g
Trisodium citrate·2H <sub>2</sub> O .....	3.0g
KCl .....	2.0g
Trace metals solution .....	0.1mL

**Trace Metals Solution:****Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.32g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.34g
Fe(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ·7H <sub>2</sub> O .....	0.78g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.14g

**Preparation of Trace Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halobacterium halobium* and *Halobacterium salinarium*.

**Halobacterium/Halococcus Medium****Composition per liter:**

Solution A .....	500.0mL
Salt solution .....	500.0mL

**Solution A:****Composition per 500.0mL:**

Skim milk .....	50.0g
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**Preparation of Solution A:** Add 50.0g of skim milk to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Salt Solution:****Composition per 500.0mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0g
KNO <sub>3</sub> .....	2.0g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloarcula vallismortis*, *Halobacterium cutirubrum*, *Halobacterium halobium*, *Halobacterium saccharovorum*, *Halobacterium salinarium*, and *Halococcus morrhuae*.

**Halobacterium Medium****Composition per liter:**

Solution 1 .....	500.0mL
Solution 2 .....	500.0mL

pH 7.0 ± 0.2 at 25°C

**Solution 1:****Composition per 500.0mL:**

Yeast extract .....	10.0g
Pancreatic digest of casein .....	2.5g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 2:****Composition per 500.0mL:**

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0g
KCl .....	5.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine sterile solution 1 and sterile solution 2. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Halobacterium salinarium*.

**Halobacterium Medium  
(ATCC Medium 974)****Composition per 100.0mL:**

Solution 1 .....	75.0mL
Solution 2 .....	25.0mL

pH 6.8 ± 0.2 at 25°C

**Solution 1:****Composition per 75.0mL:**

NaCl .....	12.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0g
K <sub>2</sub> SO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 75.0mL. Mix thoroughly. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 2:****Composition per 25.0mL:**

Agar .....	2.0g
Pancreatic digest of casein .....	0.5g
Yeast extract .....	0.5g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine sterile solution 1 and sterile solution 2. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Haloferax volcanii*.

**Halobacterium Medium  
(ATCC Medium 1176)****Composition per liter:**

NaCl .....	156.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	13.0g
Yeast extract .....	5.0g
KCl .....	4.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
Glucose .....	1.0g
NaBr .....	0.5g
NaHCO <sub>3</sub> .....	0.2g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloferax mediterranei*.

**Halobacterium Medium  
(ATCC Medium 1270)****Composition per liter:**

NaCl .....	194.0g
MgSO <sub>4</sub> .....	24.0g
MgCl <sub>2</sub> .....	16.0g
KCl .....	5.0g
Yeast extract .....	5.0g
CaCl <sub>2</sub> .....	1.0g
NaBr .....	0.5g
NaHCO <sub>3</sub> .....	0.2g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloarcula hispanica* and *Haloferax gibbonsii*.

***Halobacterium pharaonis* Medium****Composition per liter:**

NaCl .....	250.0g
Agar .....	20.0g
Casamino acids .....	15.0g
Trisodium citrate·2H <sub>2</sub> O .....	3.0g
Glutamic acid .....	2.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.5g
KCl .....	2.0g

pH 8.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Readjust pH to 8.5. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Natronobacterium* (*Halobacterium*) *pharaonis*.

***Halobacterium saccharovororum* Medium****Composition per liter:**

NaCl .....	250.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0g
Glucose .....	10.0g
Casimino acids .....	5.0g
Yeast extract .....	2.5g
KCl .....	2.0g
CaCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.2g

pH 7.35 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halobacterium saccharovororum*.

***Halobacterium soldomense* Medium****Composition per liter:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	160.0g
NaCl .....	125.0g
K <sub>2</sub> SO <sub>4</sub> .....	5.0g
Soluble starch .....	2.0g
Peptone .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.13g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halobacterium soldomense*.

***Halobacterium* Starch Medium****Composition per liter:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	160.0g
NaCl .....	125.0g
K <sub>2</sub> SO <sub>4</sub> .....	5.0g
Soluble starch .....	2.0g
Peptone .....	1.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.13g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Haloarcula marismortui*.

***Halobacterium volcanii* Medium****Composition per 100.0mL:**

NaCl .....	25.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
KCl .....	0.5g
Glycine .....	0.2g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
Yeast autolysate .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the specific enrichment of *Halobacterium volcanii*.

***Halobacteroides acetoethylicus* Medium****Composition per liter:**

NaCl .....	100.0g
Pancreatic digest of casein .....	10.0g
Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Glucose solution .....	25.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O (10% solution) .....	10.0mL
Trace elements solution .....	9.0mL
Vitamin solution .....	5.0mL
Resazurin (0.025% solution) .....	4.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (10% solution) .....	0.03mL

pH 7.3 ± 0.2 at 25°C

**Glucose Solution:****Composition per 100.0mL:**

Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Trace Elements Solution:****Composition per liter:**

Nitrilotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium DL-pantothenate .....	5.0mg

<i>p</i> -Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. While still hot, aseptically add 25.0mL of the sterile glucose solution under 97% N<sub>2</sub> + 3% H<sub>2</sub>. Adjust pH to 7.3 if necessary. Aseptically and anaerobically distribute into tubes. Cap with rubber stoppers.

**Use:** For the cultivation and maintenance of *Halobacteroides acetothylicus*.

### *Halobacteroides/Haloicola* Medium

**Composition per liter:**

NaCl.....	100.0g
Peptone.....	5.0g
CaCl <sub>2</sub> .....	0.33g
KCl.....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> .....	0.33g
NH <sub>4</sub> Cl.....	0.33g
Resazurin.....	2.0mg
Glucose solution.....	30.0mL
NaHCO <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Wolfe's vitamin solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.5 ± 0.2 at 25°C	

**Glucose Solution:**

**Composition per 30.0mL:**

D-Glucose.....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Wolfe's Vitamin Solution:**

**Composition per liter:**

Pyridoxine·HCl.....	10.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine·HCl.....	5.0mg
Calcium DL-pantothenate.....	5.0mg

Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-10:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except glucose solution, NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 30.0mL of sterile glucose solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. Check that final pH is 7.5.

**Use:** For the cultivation of *Haloanaerobium (Haloicola) saccharolyticum*.

### *Halobacteroides* Medium

**Composition per 990.0mL:**

NaCl.....	88.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	20.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	7.4g
Yeast extract.....	5.0g
KCl.....	3.7g
L-Cysteine·HCl·H <sub>2</sub> O.....	0.5g
Resazurin.....	1.0mg
Glucose (10% solution).....	50.0mL
Sodium PIPES	
(Piperazine- <i>N,N'</i> -bis-2-ethane	
sulfonate buffer, 1M, pH 6.8–7.0).....	40.0mL
pH 6.8–7.0 at 25°C	

**Preparation of Medium:** Filter sterilize glucose solution and PIPES buffer solution separately. Add remaining components—except glucose solution, PIPES buffer solution, and L-cysteine·HCl·H<sub>2</sub>O—to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling under 100% N<sub>2</sub>. Add L-cysteine·HCl·H<sub>2</sub>O. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 50.0mL of sterile glucose solution and 40.0mL of sterile PIPES buffer solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Halobacteroides halobius* and *Sporohalobacter marismortui*.

***Halobacteroides* Medium****Composition per liter:**

NaCl .....	150.0g
Sucrose .....	5.0
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0g
NaHCO <sub>3</sub> .....	2.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
NH <sub>4</sub> Cl .....	0.33g
Resazurin .....	2.0mg
Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 10% CO<sub>2</sub> + 10% H<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 10% CO<sub>2</sub> + 10% H<sub>2</sub>. Add NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Adjust pH to 7.0. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloanaerobacter lacunaris*.

***Halobaculum gomorrense* Medium  
(DSMZ Medium 823)****Composition per liter:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	160.0g
NaCl .....	125.0g
K <sub>2</sub> SO <sub>4</sub> .....	5.0g
Starch .....	2.0g
Yeast extract .....	1.0g
Casamino acids .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halobaculum gomorrense* (*Haloferrax gomorrae*).

***Halobius* Medium****Composition per liter:**

NaCl .....	116.0g
Agar .....	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Yeast extract .....	10.0g
Vitamin-free casamino acids .....	7.5g
Sodium citrate .....	3.0g
KCl .....	2.0g
FeCl <sub>2</sub> .....	0.023g

pH 6.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Micrococcus halobius*.

***Halocella cellulolytica* Medium****Composition per liter:**

NaCl .....	150.0g
Cellobiose or microcrystalline cellulose .....	5.0g
Yeast extract .....	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.6g
CaCl <sub>2</sub> .....	0.33g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
NH <sub>4</sub> Cl .....	0.33g
Resazurin .....	2.0mg
NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL.

Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Wolfe's Vitamin Solution:

#### Composition per liter:

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

### Trace Elements Solution SL-10:

#### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. Check that final pH is 7.0.

**Use:** For the cultivation of *Halocella cellulolytica*.

### Halococcus Agar

#### Composition per liter:

Solution A .....	500.0mL
Solution B .....	100.0mL
Solution C .....	400.0mL

pH 8.4 ± 0.2 at 25°C

#### Solution A:

##### Composition per 500.0mL:

Skim milk powder .....	50.0g
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**Preparation of Solution A:** Add skim milk powder to distilled/deionized water and bring volume to 500.0mL. Autoclave for 15 min at 8 psi pressure–112°C. Cool to 50°–55°C.

#### Solution B:

##### Composition per 100.0mL:

NaCl .....	200.0g
KNO <sub>3</sub> .....	2.0g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
Ferric citrate .....	1.0μg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Solution C:

##### Composition per 400.0mL:

Agar .....	25.0g
Glycerol .....	10.0g
Neopeptone .....	5.0g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 400.0mL. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 100.0mL of sterile solution B with 400.0mL of sterile solution C. Mix thoroughly. Adjust pH to 8.4. Aseptically add 500.0mL of sterile solution A. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Halococcus morrhuae*.

### Halococcus dombrowskii Medium (DSMZ Medium 954)

#### Composition per liter:

NaCl .....	200.0g
Agar .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0g
TRIS .....	12.1g
Casamino acids .....	5.0g
Yeast extract .....	5.0g
KCl .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Halococcus dombrowskii*.

### Halodurans Medium

#### Composition per liter:

NaCl .....	150.0g
Agar .....	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Sodium citrate .....	3.0g
KCl .....	2.0g
FeCl <sub>2</sub> .....	0.023g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Micrococcus varians*.



***Haloferax mediterranei* Medium****Composition per liter:**

NaCl .....	195.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	49.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	34.6g
Yeast extract .....	5.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.92g
NaBr .....	0.58g
KCl .....	0.5g
NaHCO <sub>3</sub> .....	0.17g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloferax mediterranei*.

***Haloferax mediterranei*  
Minimal Medium I****Composition per liter:**

NaCl .....	156.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	13.0g
Glucose .....	10.0g
KCl .....	4.0g
NaH <sub>2</sub> Cl .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
NaBr .....	0.58g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaHCO <sub>3</sub> .....	0.2g
FeCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.005g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloferax mediterranei*.

***Haloferax mediterranei*  
Minimal Medium II****Composition per liter:**

NaCl .....	160.0g
MgCl <sub>2</sub> ·7H <sub>2</sub> O .....	20.0g
Sodium glutamate·H <sub>2</sub> O .....	20.0g
Sucrose .....	10.0g
KCl .....	4.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloferax mediterranei*.

***Haloferax volcanii* Low-Salt Medium****Composition per liter:**

NaCl .....	125.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	45.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0g
KCl .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0g
Pancreatic digest of casein .....	3.0g
Yeast extract .....	1.34g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloferax volcanii*.

***Haloferax volcanii* Medium****Composition per liter:**

NaCl .....	206.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	37.0g
KCl .....	3.7g
Yeast tryptone solution .....	100.0mL
Tris[hydroxymethyl]aminomethane·HCl (1M solution, pH 7.2) .....	50.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution .....	5.0mL
MnCl <sub>2</sub> solution .....	1.7mL

pH 7.2 ± 0.2 at 25°C

**Yeast Tryptone Solution:****Composition per 100.0mL:**

Tryptone .....	5.0g
Yeast extract .....	3.0g

**Preparation of Yeast Tryptone Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:****Composition per 100.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0g
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**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**MnCl<sub>2</sub>·6H<sub>2</sub>O Solution:****Composition per liter:**

MnCl <sub>2</sub> ·6H <sub>2</sub> O .....	75.0mg
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**Preparation of MnCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add MnCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloferax volcanii*.

***Haloferax volcanii* Minimal Medium****Composition per liter:**

NaCl .....	206.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	36.9g
Glycerol .....	45.0mL
Sodium succinate solution .....	5.0mL
KCl solution .....	5.0mL
CaCl <sub>2</sub> solution .....	5.0mL
NH <sub>4</sub> Cl solution .....	5.0mL
MnCl <sub>2</sub> solution .....	1.7mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	2.0mL
Trace elements solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Sodium Succinate Solution:****Composition per 100.0mL:**

Sodium succinate .....	10.0g
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**Preparation of Sodium Succinate Solution:** Add sodium succinate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**KCl Solution:****Composition per 100.0mL:**

KCl .....	7.45g
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**Preparation of KCl Solution:** Add KCl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:**

**Composition** per 100.0mL:

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 10.0g

**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**NH<sub>4</sub>Cl Solution:**

**Composition** per 100.0mL:

NH<sub>4</sub>Cl ..... 5.35g

**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**MnCl<sub>2</sub>·6H<sub>2</sub>O Solution:**

**Composition** per liter:

MnCl<sub>2</sub>·6H<sub>2</sub>O ..... 75.0mg

**Preparation of MnCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add MnCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**K<sub>2</sub>HPO<sub>4</sub> Solution:**

**Composition** per 100.0mL:

K<sub>2</sub>HPO<sub>4</sub> ..... 8.7g

**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.0.

**Trace Elements Solution:**

**Composition** per 100.0mL:

FeSO<sub>4</sub> ..... 334.0mg  
MnCl<sub>2</sub> ..... 36.0mg  
ZnSO<sub>4</sub> ..... 44.0mg  
CuSO<sub>4</sub> ..... 5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except CaCl<sub>2</sub> solution, to distilled/deionized water and bring volume to 995.0mL. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 5.0mL of sterile CaCl<sub>2</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Haloferax volcanii*.

**Halomethanococcus Medium**  
(*Methanohalophilus* Medium)

**Composition** per 1030.0mL:

Trimethylamine-HCl ..... 2.5g  
Na<sub>2</sub>CO<sub>3</sub> ..... 2.0g  
NaHCO<sub>3</sub> ..... 2.0g  
Casamino acids ..... 0.5g  
L-Cysteine-HCl·H<sub>2</sub>O ..... 0.5g  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 0.5g  
NH<sub>4</sub>Cl ..... 0.5g  
Pancreatic digest of casein ..... 0.5g  
Yeast extract ..... 0.5g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.2g  
Ammonium-2-mercaptoethanesulfonate ..... 1.0mg  
Artificial brine ..... 1.0L  
Wolfe's vitamin solution ..... 10.0mL

Wolfe's mineral solution ..... 10.0mL  
Volatile acids solution ..... 10.0mL  
pH 7.1 ± 0.2 at 25°C

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 2.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Artificial Brine:**

**Composition** per liter:

NaCl ..... 80.7g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 35.1g  
Na<sub>2</sub>SO<sub>4</sub> ..... 12.9g  
KCl ..... 5.7g  
CaCl<sub>2</sub> ..... 0.55g  
LiCl ..... 0.13g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.12g

**Preparation of Artificial Brine:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Wolfe's Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl ..... 0.01g  
Thiamine-HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Thioctic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Store at 4°C.

**Wolfe's Mineral Solution:**

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitriloacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.5g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub> ..... 0.1g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
AlK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.01g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Volatile Acids Solution:**

**Composition** per liter:

α-Methylbutyric acid ..... 0.5mL  
Isobutyric acid ..... 0.5mL  
Isovaleric acid ..... 0.5mL  
Valeric acid ..... 0.5mL

**Preparation of Volatile Acids Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components, except the L-cysteine-HCl-H<sub>2</sub>O, trimethylamine-HCl, and Na<sub>2</sub>S-9H<sub>2</sub>O solution. Mix thoroughly. Gently heat and bring to boiling. Add L-cysteine-HCl-H<sub>2</sub>O. Mix thoroughly. Cool in an ice-water bath under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add trimethylamine-HCl. Mix thoroughly. Adjust pH to 7.1. Aseptically and anaerobically distribute into tubes in 10.0mL volumes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure-121°C. Immediately prior to inoculation, aseptically add 0.25mL of sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution to each tube. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Methanohalophilus mahii*.

#### *Halomonas desiderata* Medium (DSMZ Medium 762)

##### Composition per liter:

Glucose .....	5.0g
KNO <sub>3</sub> .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Carbonate solution .....	100.0mL
pH 9.5-10.0 at 25°C	

##### Carbonate Solution

##### Composition per 100mL:

Na <sub>2</sub> CO <sub>3</sub> .....	5.4g
NaHCO <sub>3</sub> .....	4.2g

**Preparation of Carbonate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbonate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C. Cool to room temperature. Aseptically add 100.0mL sterile carbonate solution. Mix thoroughly. Aseptically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Halomonas desiderata*.

#### *Halomonas magadiensis* Medium (DSMZ Medium 971)

##### Composition per liter:

Agar .....	20.0g
Glucose .....	10.0g
Peptone .....	5.0g
Yeast extract .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl solution .....	200.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	100.0mL
pH 10.0 ± 0.2 at 25°C	

##### NaCl Solution:

##### Composition per 200.0mL:

NaCl .....	40.0g
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**Preparation of NaCl Solution:** Add NaCl to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 60°C.

##### Na<sub>2</sub>CO<sub>3</sub> Solution:

##### Composition per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	10.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 60°C.

**Preparation of Medium:** Add components, except NaCl solution and Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 700.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 60°C. Aseptically add 200.0mL NaCl solution and 100.0mL Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Halomonas magadiensis*.

#### *Halomonas* Medium

##### Composition per liter:

NaCl .....	80.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Casamino acids with vitamins .....	7.5g
Proteose peptone No. 3 .....	5.0g
Sodium citrate .....	3.0g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.05g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation and maintenance of *Halomonas elongata*.

#### *Halomonas pantelleriense* Agar (DSMZ Medium 752)

##### Composition per liter:

NaCl .....	100.0g
Agar .....	20.0g
Yeast extract .....	10.0g
Na <sub>2</sub> -citrate .....	3.0g
KCl .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Na <sub>2</sub> CO <sub>3</sub> solution .....	25.0mL
MnCl <sub>2</sub> solution .....	1.0mL
FeSO <sub>4</sub> solution .....	1.0mL
pH 9.0 ± 0.2 at 25°C	

##### Na<sub>2</sub>CO<sub>3</sub> Solution:

##### Composition per 25.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	2.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly.

##### FeSO<sub>4</sub> Solution:

##### Composition per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
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**Preparation of FeSO<sub>4</sub> Solution:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

##### MnCl<sub>2</sub> Solution:

##### Composition per 100.0mL:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.036g
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**Preparation of MnCl<sub>2</sub> Solution:** Add MnCl<sub>2</sub>·4H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution to distilled/deionized water and bring volume to 990.0L. Mix thoroughly. Adjust pH to 9.0. Autoclave for 15 min at 15 psi pressure-121°C. Aseptically add

10.0mL sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Pour into Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Halomonas pantelleriensis* (*Halomonas pantelleriense*).

### ***Halomonas subglaciescola* Medium (Artificial Organic Lake Medium)**

**Composition** per 1001.0mL:

NaCl .....	80.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.5g
Yeast extract .....	1.0g
KCl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
KNO <sub>3</sub> .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
Hutner's basal salts solution .....	20.0mL
Phosphate supplement .....	20.0mL
Vitamin solution .....	1.0mL

pH 8.0 ± 0.2 at 25°C

### **Hutner's Basal Salts Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>7</sub> ·O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44" .....	50.0mL

### **"Metals 44":**

**Composition** per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8. Filter sterilize.

### **Phosphate Supplement:**

**Composition** per 20.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	50.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	50.0mg

**Preparation of Phosphate Supplement:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

### **Vitamin Solution:**

**Composition** per liter:

Cyanocobalamin .....	10.0mg
Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Nicotinamide .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Hutner's basal salts solution, phosphate supplement, and vitamin solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Adjust pH to 8.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile Hutner's basal salts solution, 20.0mL of sterile phosphate supplement, and 1.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Halomonas subglaciescola*.

### ***Halonatronum saccharophilum* Medium (DSMZ Medium 932)**

**Composition** per liter:

Na <sub>2</sub> CO <sub>3</sub> .....	68.0g
NaCl .....	50.0g
NaHCO <sub>3</sub> .....	38.0g
NH <sub>4</sub> Cl .....	0.5g
KCl .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgCl <sub>2</sub> .....	0.1g
Resazurin .....	0.01g
Sucrose solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Yeast extract solution .....	10.0mL
Vitamin solution .....	10.0mL
Trace elements .....	1.0mL

pH 9.5–10.0 at 25°C

### **Sucrose Solution:**

**Composition** per 50.0mL:

Sucrose .....	5.0g
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**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### **Yeast Extract Solution:**

**Composition** per 10.0mL:

Yeast extract .....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.7g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### **Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub>, NH<sub>4</sub>Cl, Na<sub>2</sub>CO<sub>3</sub>, sucrose solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, yeast extract solution, and vitamin solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add solid NaHCO<sub>3</sub>, NH<sub>4</sub>Cl, and Na<sub>2</sub>CO<sub>3</sub>. Mix thoroughly. Distribute into anaerobe tubes or bottles. Aseptically and anaerobically add per liter of medium 50.0mL sucrose solution, 10.0mL yeast extract solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL vitamin solution. Final pH 9.5-10.0. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Halonatronum saccharophilum*.

#### Halophile Agar

##### Composition per liter:

NaCl .....	156.0g
Agar .....	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	13.0g
Yeast extract .....	10.0g
KCl .....	4.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
NaBr .....	0.5g
NaHCO <sub>3</sub> .....	0.2g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Haloarcula hispanica*, *Haloferax gibbonsii*, *Marinococcus halophilus*, *Planococcus* species, and *Sporosarcina halophila*.

#### Halophile Agar I

##### Composition per liter:

NaCl .....	250.0g
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MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Agar .....	15.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Trisodium citrate .....	3.0g
KCl .....	2.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.25mg

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Add agar. Gently heat and bring to boiling. Do not autoclave. Sterilize by steaming at 100°C for 30 min. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Halobacterium halobium*, *Halobacterium salinarum*, and *Halobacterium trapanicum*.

#### Halophile Medium

##### Composition per liter:

NaCl .....	30.0g
Agar .....	15.0g
Peptone .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus tusciae*, *Chromobacterium maris-mortui*, *Flavobacterium tirrenicum*, *Flavobacterium uliginosum*, *Halomonas halomphila*, *Pseudomonas beijerinckii*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, and *Vibrio proteolyticus*.

#### Halophile Medium

##### Composition per liter:

NaCl .....	100.0g
KCl .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
NH <sub>4</sub> Cl .....	5.0g
Peptone solution (15% solution) .....	30.0mL
Yeast extract solution (15% solution) .....	30.0mL
Ferric citrate solution (1% solution) .....	10.0mL
Trace elements solution .....	5.0mL

##### Trace Elements Solution:

##### Composition per liter:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22g
MgCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.18g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	6.3mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Rhodospirillum salinarum*.

**Halophile Medium III****Composition per liter:**

NaCl .....	95.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	81.3g
Glycerol .....	5.0g
NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> solution .....	100.0mL
Wolfe's vitamin solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.5 ± 0.2 at 25°C	

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium D-(+)-pantothenate .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid, .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**K<sub>2</sub>HPO<sub>4</sub> Solution:****Composition per 100.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C

**Preparation of Medium:** Add components, except K<sub>2</sub>HPO<sub>4</sub> solution, Wolfe's vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 889.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 1000.0mL of sterile K<sub>2</sub>HPO<sub>4</sub> solution, 10.0mL of sterile Wolfe's vitamin solution, and 1.0mL of sterile trace elements solution SL-10. Mix thoroughly. Aseptically adjust pH to 7.5. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Halovibrio variabilis*.

**Halophilic Agar  
(HA)****Composition per liter:**

NaCl .....	250.0g
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MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	25.0g
Agar .....	20.0g
Casamino acids .....	10.0g
Yeast extract .....	10.0g
Proteose peptone .....	5.0g
Trisodium citrate .....	3.0g
KCl .....	2.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Combine the ingredients with distilled water and heat to boiling to dissolve completely. Autoclave at 121°C for 15 min.

**Use:** For the isolation and cultivation of halophilic microorganisms, such as *Pseudomonas* species and *Flavobacterium* species, from fish.

**Halophilic Broth  
(HB)****Composition per liter:**

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	25.0g
Casamino acids .....	10.0g
Yeast extract .....	10.0g
Proteose peptone .....	5.0g
Trisodium citrate .....	3.0g
KCl .....	2.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of halophilic microorganisms, such as *Pseudomonas* species and *Flavobacterium* species, from fish.

**Halophilic Chromatium Medium****Composition per 1060.0mL:**

NaCl .....	60.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Solution A .....	20.0mL
Solution B .....	20.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL
Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of Solution A:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution B:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition per 100.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution C:** Add  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution D:

**Composition** per 100.0mL:

Sodium malate ..... 10.0g

**Preparation of Solution D:** Add sodium malate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution:

**Composition** per liter:

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  ..... 2.7g  
 $\text{H}_3\text{BO}_3$  ..... 0.1g  
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g  
 $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  ..... 50.0mg  
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 5.0mg  
 $\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except solution A, solution B, solution C, and solution D, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 7.0–7.2 with  $\text{H}_3\text{PO}_4$ . Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.2mL of sterile solution A, 0.2mL of sterile solution B, 0.1mL of sterile solution C, and 0.1mL of sterile solution D for each 10.0mL of medium. Mix thoroughly. Use immediately.

**Use:** For the cultivation of halophilic *Chromatium* species.

### Halophilic Clostridium Agar

**Composition** per liter:

L-Cysteine-HCl-H<sub>2</sub>O ..... 0.5g  
 Solution 1 ..... 1.0L  
 Solution 2 ..... 100.0mL

pH 6.2–7.0 at 25°C

#### Solution 1:

**Composition** per liter:

NaCl ..... 105.0g  
 Agar ..... 20.0g  
 KCl ..... 7.5g  
 $\text{CaCO}_3$  ..... 5.0g  
 L-Glutamic acid ..... 4.0g  
 Soluble starch ..... 2.0g  
 Casamino acids ..... 2.0g  
 Nutrient broth ..... 2.0g  
 Yeast extract ..... 2.0g  
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 2.0mg  
 Resazurin ..... 1.0mg  
 NaOH (2.5N solution) ..... 12.5mL  
 Wolfe's vitamin solution ..... 10.0mL  
 Wolfe's mineral solution ..... 10.0mL

**Preparation of Solution 1:** Add components, except  $\text{CaCO}_3$ , to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. When all components have dissolved, add the  $\text{CaCO}_3$ . Mix thoroughly.

#### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl ..... 0.01g  
 Thiamine-HCl ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Nicotinic acid ..... 5.0mg

Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Thioctic acid ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Cyanocobalamin ..... 0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Wolfe's Mineral Solution:

**Composition** per liter

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 3.0g  
 Nitroloacetic acid ..... 1.5g  
 NaCl ..... 1.0g  
 $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  ..... 0.5g  
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g  
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 0.1g  
 $\text{CaCl}_2$  ..... 0.1g  
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g  
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 0.01g  
 $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  ..... 0.01g  
 $\text{H}_3\text{BO}_3$  ..... 0.01g  
 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitroloacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### Solution 2:

**Composition** per 100.0mL:

$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 20.3g  
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 7.35g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gas with 100%  $\text{N}_2$  for 20 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Gently heat 1.0L of solution 1 and bring to boiling under 100%  $\text{N}_2$ . Add the L-cysteine-HCl-H<sub>2</sub>O. Continue boiling until resazurin turns colorless, indicating reduction. The volume of solution 1 should be about 900.0mL. Anaerobically distribute into tubes in 9.0mL volumes under 100%  $\text{N}_2$ . Cap tubes with rubber stoppers. Place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust. Cool to 50°C. Aseptically add 1.0mL of sterile solution 2 to each tube. In the presence of  $\text{CaCO}_3$ , the pH may be higher than 7.0. Do not adjust pH.

**Use:** For the cultivation and maintenance of *Sporohalobacter lortetii*.

### Halophilic Halobacterium Medium

**Composition** per liter:

NaCl ..... 200.0g  
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 37.0g  
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 0.7g  
 KCl ..... 0.5g  
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 0.05g  
 Yeast extract ..... 100.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of extremely halophilic *Halobacterium* species.

### Halophilic Nutrient Agar (LMG Medium 220)

**Composition** per liter:

NaCl .....	60.0g
Agar .....	15.0g
Casein peptone, tryptic digest .....	10.0g
Yeast extract .....	5.0g
Glucose .....	5.0g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of halophilic bacteria.

### Halophilic Synthetic Medium

**Composition** per liter:

Glucose .....	0.1g
KNO <sub>3</sub> .....	0.05g
FePO <sub>4</sub> .....	0.01g
Artificial seawater .....	100.0mL

### Artificial Seawater:

**Composition** per 100.0mL:

NaCl .....	2.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.1g
Na <sub>2</sub> SO <sub>4</sub> .....	0.4g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
KCl .....	0.07g
NaHCO <sub>3</sub> .....	0.02g
KBr .....	0.01g
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0mg
H <sub>3</sub> BO <sub>3</sub> .....	3.0mg
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O .....	0.5mg
NaF .....	0.3mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of halophilic bacteria.

### Halorhabdus utahensis Medium (DSMZ Medium 927)

**Composition** per 1003.25mL:

NaCl .....	270.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Tris-HCl .....	12.0g
KCl .....	5.0g
NH <sub>4</sub> Cl .....	2.0g
Glucose .....	2.0g
Yeast extract .....	1.0g
NaBr .....	0.1g
Phosphate solution .....	2.5mL
Calcium chloride solution .....	0.5mL
Iron chloride manganese chloride solution .....	0.25mL

pH 7.6 ± 0.2 at 25°C

### Potassium Phosphate Solution:

**Composition** per 10.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
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### Preparation of Potassium Phosphate Solution:

Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Calcium Chloride Solution:

**Composition** per 10.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
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### Preparation of Calcium Chloride Solution:

Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Iron Chloride Manganese Chloride Solution:

**Composition** per 10.0mL:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2g

### Preparation of Iron Chloride Manganese Chloride Solution:

Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except potassium phosphate solution, calcium chloride solution, and iron chloride manganese chloride solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.6 with 5M NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 2.5mL phosphate solution, 0.5mL calcium chloride solution, and 0.25mL iron chloride manganese chloride solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Halorhabdus utahensis*.

### Halothermothrix orenii Medium (DSMZ Medium 761)

**Composition** per liter:

NaCl .....	100.0g
Glucose .....	10.0g
KCl .....	4.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Na-acetate .....	1.0g
Trypticase™ .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
Resazurin .....	0.001g
NaHCO <sub>3</sub> solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Dithionite solution .....	5.0mL
Trace elements SL-6 .....	1.0mL

pH 7.0 ± 0.2 at 25°C

### Trace Elements Solution SL-6:

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g



**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.2g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Neutralize to pH 7.0 with sterile HCl.

#### **NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 10.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### **Dithionite Solution:**

**Composition** per 10mL:

Na-dithionite ..... 2.0mg

**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, dithionite solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 935.0mL. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Cool while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into Hungate tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically inject NaHCO<sub>3</sub> solution (0.5mL/10mL medium), dithionite solution (0.05mL/10mL medium), and Na<sub>2</sub>S·9H<sub>2</sub>O solution (0.1mL/10mL medium). Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Halothermothrix orenii*.

### ***Halothiobacillus* Medium (DSMZ Medium 864)**

**Composition** per 1030mL:

NaCl ..... 29.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.5g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0g  
KCl ..... 0.7g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.42g  
Bromothymol Blue ..... 4.0mg  
Na-thiosulfate solution ..... 20.0mL  
Phosphate solution ..... 10.0mL  
Trace elements solution ..... 1.0mL

pH 6.5–7.0 at 25°C

#### **Trace Elements Solution:**

**Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
Na<sub>2</sub>-EDTA ..... 0.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg

CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 7.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na-thiosulfate Solution:**

**Composition** per 20.0mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 5.0g

**Preparation of Na-thiosulfate Solution:** Add 5.0g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### **Phosphate Solution:**

**Composition** per 10.0mL:

K<sub>2</sub>HPO<sub>4</sub> ..... 0.5g

**Preparation of Phosphate Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except phosphate solution and Na-thiosulfate solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.7. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 20.0mL sterile Na-thiosulfate solution and 10.0mL sterile phosphate solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Halothiobacillus kellyi*.

### ***Halovibrio variabilis* Medium**

**Composition** per liter:

NaCl ..... 95.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 81.0g  
Yeast extract ..... 7.5g  
Proteose peptone ..... 2.5g  
KCl ..... 1.0g  
Trace elements solution SL-4 ..... 10.0mL  
Vitamin solution ..... 10.0mL

pH 7.5 ± 0.2 at 25°C

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine HCl ..... 1.0mg  
Lipoic acid ..... 0.5mg  
Nicotinic acid ..... 0.5mg  
*p*-Aminobenzoic acid ..... 0.5mg  
Pantothenic acid ..... 0.5mg  
Riboflavin ..... 0.5mg  
Thiamine HCl ..... 0.5mg  
Biotin ..... 0.2mg  
Folic acid ..... 0.2mg  
Cyanocobalamin ..... 0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Store at 5°C.

#### **Trace Elements Solution SL-4:**

**Composition** per 900.0mL:

EDTA ..... 0.5g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
Trace elements solution SL-6 ..... 100.0mL

**Trace Elements Solution SL-6:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except trace elements solution SL-4 and vitamin solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile trace elements solution SL-4 and 10.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Halovibrio variabilis*.

**Harpo's Htye****Composition** per liter:

Pancreatic digest of casein.....	5.0g
HEPES ( <i>N</i> -[2-Hydroxyethyl]piperazine- <i>N'</i> -2-ethanesulfonic acid) buffer.....	4.0g
Yeast extract.....	2.0g

pH 6.8–7.0 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0–7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Cytophaga arvensicola* and *Flexibacter columnaris*.

**Harpo's HTYEM Marine Medium**

Pancreatic digest of casein.....	5.0g
HEPES.....	4.0g
Yeast extract.....	2.0g
Artificial seawater.....	1.0L

pH 7.5 ± 0.2 at 25°C

**Artificial Seawater:****Composition** per liter:

NaCl.....	27.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.78g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.38g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.4g
KCl.....	0.72g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to artificial seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Cytophaga fermentans*, *Cytophaga latercula*, *Cytophaga uliginosa*, and *Microscilla agregans*.

**Hay Infusion Agar****Composition** per liter:

Hay, partially decomposed.....	50.0g
Agar.....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g

pH 6.2 ± 0.3 at 25°C

**Preparation of Medium:** Add hay to distilled/deionized water and bring volume to 1.0L. Autoclave for 30 min at 15 psi pressure–121°C. Filter through paper and reserve filtrate. Add distilled/deionized water to filtrate and bring volume to 1.0L. Mix thoroughly. Add agar and K<sub>2</sub>HPO<sub>4</sub>. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alternaria* species, *Caulochytrium protostelioides*, *Choanephora infundibulifera*, *Dipsacomycetes acuminosporus*, *Eremascus albus*, *Eremascus fertilis*, *Eurotium chevalieri*, *Eurotium halophilicum*, *Eurotium herbariorum*, *Mortierella bisporalis*, *Protostelium irregularis*, and *Saksenaeva vasisformis*.

**HE Medium  
(Hay Extract Medium)****Composition** per liter:

Agar.....	10.0g
Peptone.....	1.0g
Yeast extract.....	1.0g
Hay extract solution.....	500.0mL

pH 6.5 ± 0.2 at 25°C

**Hay Extract Solution:****Composition** per liter:

Hay, dried.....	50.0g
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**Preparation of Hay Extract Solution:** Add dried barn hay to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Filter through Whatman #40 filter paper.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Spirochaeta aurantia*.

**Heart Infusion Agar****Composition** per liter:

Beef heart, infusion from.....	500.0g
Agar.....	15.0g
Tryptose.....	10.0g
NaCl.....	5.0g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of a wide variety of fastidious microorganisms. For the cultivation and maintenance of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus mycoides*, *Serratia rubidaea*, *Staphylococcus aureus*, *Tsatumella ptyseos*, and *Vibrio vulnificus*.

**Hektoen Enteric Agar****Composition per liter:**

Agar .....	13.5g
Lactose .....	12.0g
Peptic digest of animal tissue .....	12.0g
Sucrose .....	12.0g
Bile salts .....	9.0g
NaCl .....	5.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	5.0g
Yeast extract .....	3.0g
Salicin .....	2.0g
Ferric ammonium citrate .....	1.5g
Acid Fuchsin .....	0.1g
Bromothymol Blue .....	0.064g

pH 7.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems, and Oxoid Unipath.

**Caution:** Acid Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contact with the skin.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring until components are dissolved. Do not autoclave. Pour into sterile Petri dishes. Allow agar to solidify with the Petri dish covers partially off.

**Use:** For the isolation and differentiation of *Salmonella* and *Shigella*. Bacteria that ferment lactose or sucrose appear as yellow to orange colonies. Bacteria that produce H<sub>2</sub>S appear as colonies with black centers.

***Heliobacillus mobilis* Medium****Composition per 966.0mL:**

Yeast extract .....	10.0g
MgSO <sub>4</sub> .....	0.1g
EDTA .....	2.0mg
Sodium pyruvate solution .....	100.0mL
Trace elements solution B .....	10.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	5.0mL
Trace elements solution A .....	1.0mL

pH 7.1 ± 0.2 at 25°C

**Sodium Pyruvate Solution:****Composition per 100.0mL:**

Sodium pyruvate .....	1.1g
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**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution B:****Composition per 100.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.3g
Ferric ammonium citrate .....	0.2g

**Preparation of Trace Elements Solution B:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**K<sub>2</sub>HPO<sub>4</sub> Solution:****Composition per 100.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution A:****Composition per 100.0mL:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	49.4mg

**Preparation of Trace Elements Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except sodium pyruvate solution, trace elements solution B, K<sub>2</sub>HPO<sub>4</sub> solution, and trace elements solution A, to distilled/deionized water and bring volume to 850.0mL. Mix thoroughly. Adjust pH to 7.1. Autoclave for 15 min at 15 psi pressure—121°C. Cool to room temperature. Aseptically add 100.0mL of sterile sodium pyruvate solution, 10.0mL of sterile trace elements solution B, 5.0mL of sterile K<sub>2</sub>HPO<sub>4</sub> solution, and 1.0mL of sterile trace elements solution A. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Heliobacillus mobilis*.

***Heliobacterium chlorum* Medium****Composition per liter:**

Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Sodium ascorbate .....	0.5g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except sodium ascorbate, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Sparge with 100% N<sub>2</sub> and continue boiling for 3–4 min. Add sodium ascorbate and continue to sparge with 100% N<sub>2</sub>. Adjust pH to 6.8. Under 100% N<sub>2</sub>, immediately dispense 45.0mL of medium into 50.0mL screw-capped tubes fitted with rubber septa. Tighten screw caps. Autoclave for 15 min at 15 psi pressure—121°C.

**Use:** For the cultivation and maintenance of *Heliobacillus mobilis* and *Heliobacterium chlorum*.

***Heliorestis* Medium  
(DSMZ Medium 886)****Composition per liter:**

Na-acetate .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.6g
Yeast extract .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaCl .....	0.5g
Resazurin .....	0.2g
CaCl <sub>2</sub> .....	0.1g
Vitamin B <sub>12</sub> .....	20.0μg
Biotin .....	20.0μg
Solution A .....	50.0mL
Trace elements SL-6 .....	1.0mL

pH 9.0–9.5 at 25°C

**Solution A:****Composition per 50mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	2.5g
NaHCO <sub>3</sub> .....	2.5g
NH <sub>4</sub> Cl .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure—121°C. Cool to 25°C.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except solution A, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 50.0mL solution A. Mix thoroughly. Adjust pH to 9.0–9.5. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Heliorestis baculata*.

***Herbaspirillum* Agar****Composition per liter:**

Agar .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
NaCl .....	0.1g
Yeast extract .....	0.05g
CaCl <sub>2</sub> .....	0.02g
FeCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
Solution A .....	50.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition per 50.0mL**

Sodium malate .....	5.0g
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**Preparation of Solution A:** Add sodium malate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust pH to 7.0. Filter sterilize.

**Preparation of Medium:** Add components, except solution A, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 50.0mL of sterile solution A. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Herbaspirillum seropedicae*.

***Herpetosiphon giganteus* Medium****Composition per liter:**

Pancreatic digest of casein .....	3.0g
Yeast extract .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Herpetosiphon giganteus*.

**HESNW Medium****Composition per 1011.0mL:**

Natural seawater .....	1.0L
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Enrichment solution .....	10.0mL
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Vitamin solution .....	1.0mL
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**Enrichment Solution:****Composition per liter:**

NaNO <sub>3</sub> .....	4.667g
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O .....	3.000g
Sodium glycerophosphate .....	0.667g
EDTA·2H <sub>2</sub> O .....	0.553g
H <sub>3</sub> BO <sub>3</sub> .....	0.380g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.234g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.054g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.016g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.3mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.6mg

**Preparation of Enrichment Solution:** Add Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O to distilled/deionized water. Mix thoroughly. Neutralize Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O with 1N HCl. Add 500.0mL of distilled/deionized water. Mix thoroughly. Add remaining components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition per liter:**

Thiamine .....	0.1g
Vitamin B <sub>12</sub> .....	2.0mg
Biotin .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Allow natural seawater to age for 2 months. Filter sterilize. Aseptically add 10.0mL of sterile enrichment solution and 1.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Amphiprora hyalina*, *Chlamydomonas hedleyi*, *Chlamydomonas provasolii*, *Chlorella saccharophila*, *Chroomonas salina*, *Pavlova lutheri*, and *Trichosphaerium* species.

**Heterotrophic Hyperthermophilic Archaea Medium****Composition per liter:**

Pancreatic digest of casein .....	3.0g
Glucose .....	3.0g
Yeast extract .....	3.0g
Artificial seawater .....	990.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Artificial Seawater:****Composition per liter:**

NaCl .....	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
NaHCO <sub>3</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.3g
KCl .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.42g
NaBr .....	0.05g
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
Fe(NH <sub>4</sub> ) citrate .....	0.01g
Wolfe's mineral solution .....	5.0mL
Vitamin solution .....	5.0mL

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitritotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Vitamin Solution:****Composition per liter:**

Niacin	10.0mg
Pantothenate	10.0mg
Lipoic acid	10.0mg
<i>p</i> -Aminobenzoic acid	10.0mg
Thiamine (B <sub>1</sub> )	10.0mg
Riboflavin (B <sub>2</sub> )	10.0mg
Pyridoxine (B <sub>6</sub> )	10.0mg
Cobalamin (B <sub>12</sub> )	10.0mg
Biotin	4.0mg
Folic acid	4.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.8g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to artificial seawater and bring volume to 990.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub> for 20 min. Aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust medium pH to 7.2 by adding sterile anaerobic HCl. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of hyperthermophilic archaea.

### Heterotrophic Medium H3P (DSMZ Medium 428)

**Composition per 1010mL:**

Solution B	855.0mL
Solution A	50.0mL
Solution E	50.0mL
Solution D	30.0mL

Solution C	20.0mL
Standard vitamin solution	5.0mL

**Solution A:****Composition per 50mL:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O	2.9g
KH <sub>2</sub> PO <sub>4</sub>	2.3g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B:****Composition per 855mL:**

NH <sub>4</sub> Cl	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.010g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.005g
NaVO <sub>3</sub> ·H <sub>2</sub> O	0.005g
Trace elements solution SL-6	5.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 855.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.5g
H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Solution C:****Composition per 20mL:**

Ferric ammonium citrate	0.050g
Distilled water	20.0mL

**Preparation of Solution C:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution D:****Composition per 30mL:**

Yeast extract	1.0g
Na-acetate	1.0g
Na <sub>2</sub> -succinate	1.0g
DL-Malate	1.0g

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Adjust to pH 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution E:****Composition per 50mL:**

D-Glucose	2.0g
Na-lactate	1.0g
Na-pyruvate	1.0g
D-Mannitol	1.0g

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust to pH 7.0. Filter sterilize.

**Standard Vitamin Solution:****Composition** per 100mL:

Thiamine-HCl·2H <sub>2</sub> O	50.0mg
Nicotinic acid	50.0mg
Pyridoxine-HCl	50.0mg
Ca-pantothenate	50.0mg
Riboflavin	10.0mg
Vitamin B <sub>12</sub>	1.0mg
Folic acid	0.2mg
Biotin	0.1mg

**Preparation of Standard Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically mix solutions A, B, C, D, E, and standard vitamin solution. Aseptically distribute to flasks or tubes.

**Use:** For the cultivation and maintenance of *Bacillus* spp., *Pseudomonas* spp., *Xanthomonas campestris* pvar. *Campestris*, *Xanthobacter* sp., *Aquaspirillum arcticum*, *Herbaspirillum seropedicae*, *Sphingobium chlorophenolicum*=*Sphingomonas chlorophenolica*, and *Azoarcus* sp. Also H3P is a heterotrophic medium for growth, purity checking, and isolation of a broad spectrum of aerobic bacteria.

### Heterotrophic Medium for *Hydrogenomonas*

**Composition** per liter:

Agar	15.0g
Tryptose	5.0g
Cornstarch	2.0g
Sodium succinate·6H <sub>2</sub> O	2.0g
Sodium glutamate	1.0g
Yeast extract	1.0g
Sodium citrate·2H <sub>2</sub> O	0.5g
Sodium acetate·3H <sub>2</sub> O	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
MgSO <sub>4</sub>	0.1g

pH 6.8–7.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the heterotrophic cultivation of *Hydrogenomonas* species.

### Heterotrophic Medium for Hydrogen-Oxidizing Bacteria

**Composition** per 1010.0mL:

Solution A	900.0mL
Solution C	100.0mL
Solution B	10.0mL

**Solution A:****Composition** per 900.0mL:

Noble agar	17.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	9.0g
KH <sub>2</sub> PO <sub>4</sub>	1.5g
NH <sub>4</sub> Cl	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Trace elements solution SL-6	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution SL-6:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Solution B:****Composition** per 10.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g
Ferric ammonium citrate	5.0mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution C:****Composition** per 100.0mL:

Sodium 3-hydroxybutyrate	2.0g
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**Preparation of Solution C:** Add sodium 3-hydroxybutyrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 45°–50°C.

**Preparation of Medium:** Aseptically combine 900.0mL of sterile solution A, 10.0mL of sterile solution B, and 100.0mL of sterile solution C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the heterotrophic cultivation and maintenance of *Xanthobacter agilis*.

### Heterotrophic Nitrobacter Medium (DSMZ Medium 756)

**Composition** per liter:

Yeast extract	1.5g
Peptone	1.5g
Na-pyruvate	0.55g
Stock solution	100.0mL
Trace elements solution	1.0mL

pH 7.4 ± 0.2 at 25°C

**Stock Solution:****Composition** per liter:

NaCl	5.0g
KH <sub>2</sub> PO <sub>4</sub>	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
CaCO <sub>3</sub>	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:****Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O	97.3mg
H <sub>3</sub> BO <sub>3</sub>	49.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	43.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	37.1mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O	33.8mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O	25.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Nitrospira moscoviensis*, *Nitrobacter hamburgensis*, *Nitrobacter vulgaris*, and *Nitrobacter winogradskyi*.

### Heterotrophic *Nitrobacter* Medium (LMG Medium 245)

**Composition per liter:**

Yeast extract .....	1.5g
Peptone .....	1.5g
Na-pyruvate .....	0.55g
Stock solution .....	100.0mL
Trace elements solution .....	1.0mL

pH 7.4 ± 0.2 at 25°C

#### Stock Solution:

**Composition per liter:**

NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

**Composition per liter:**

(NH <sub>4</sub> )Mo7O <sub>2</sub> .....	437.1mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	97.3mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	43.1mg
H <sub>3</sub> BO <sub>3</sub> .....	39.4mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	33.8mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	25.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of heterotrophic *Nitrobacter* spp.

### Hexamita Medium

**Composition per liter:**

TYGM-9 medium .....	250.0mL
Sonneborn's <i>Paramecium</i> medium .....	750.0mL

#### TYGM-9 Medium:

**Composition per liter:**

NaCl .....	7.5g
K <sub>2</sub> HPO <sub>4</sub> .....	2.8g
Casein digest .....	2.0g
Gastric mucin .....	2.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
Bovine serum, heat inactivated .....	30.0mL
Rice starch solution .....	30.0mL
Tween solution .....	0.5mL

pH 7.4 ± 0.2 at 25°C

#### Tween Solution:

**Composition per 100.0mL:**

Tween 80 .....	10.0mL
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**Preparation of Tween Solution:** Add Tween 80 to absolute ethanol and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Rice Starch Solution:

**Composition per 100.0mL:**

Rice starch .....	5.0g
Phosphate buffered saline solution .....	100.0mL

#### Phosphate Buffered Saline Solution:

**Composition per liter:**

NaCl .....	9.0g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	0.795g
KH <sub>2</sub> PO <sub>4</sub> .....	0.114g

#### Preparation of Phosphate Buffered Saline Solution:

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Rice Starch Solution:** Heat sterilize rice starch at 150°C for 2 hr. Aseptically add 100.0mL of sterile phosphate-buffered saline solution. Mix thoroughly. Use immediately.

**Preparation of TYGM-9 Medium:** Add components, except rice starch solution, Tween solution, and bovine serum, to distilled/deionized water and bring volume to 939.5mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 30.0mL of sterile bovine serum, 30.0mL of sterile rice starch solution, and 0.5mL of sterile Tween solution. Mix thoroughly. Aseptically distribute into sterile, screw-capped tubes or flasks.

#### Sonneborn's *Paramecium* Medium:

**Composition per liter:**

Solution 1 .....	1.0L
<i>Klebsiella pneumoniae</i> cultured on solution 2 .....	variable

#### Solution 1:

**Composition per liter:**

Rye grass cerophyll .....	2.5g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.5g

**Source:** Cerophyll can be obtained from Ward's Natural Science Establishment, Inc. Dairy Goat Nutrition distributes Grass Media Culture, which is equivalent. Cereal Leaf Product from Sigma Chemical is similar to cerophyll.

**Preparation of Solution 1:** Add cerophyll to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boil. Boil for 5 min. Filter through Whatman #1 filter paper. Add 0.5g of Na<sub>2</sub>HPO<sub>4</sub>. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute 10.0mL volumes into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution 2:

**Composition per liter:**

Agar .....	20.0g
Yeast extract .....	4.0g
Glucose .....	0.16g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute 5.0mL into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Preparation of Sonneborn's *Paramecium* Medium:** Inoculate the surface of agar slants of solution 2 with a culture of *Klebsiella pneumoniae*. Incubate at 37°C for

24–48 hr. Scrape cells from the surface of the agar slants and add to 10.0mL of solution 1. Incubate at 30°C for 24 hr.

**Preparation of Medium:** Aseptically combine 3.0mL of sterile TYGM-9 medium with 9.0mL of Sonneborn's *Paramedium* medium in 16 × 125mm screw-capped test tubes.

**Use:** For the cultivation of *Hexamita inflata*, *Hexamita pusilla*, *Mastigamoeba invertens*, and *Trepomonas agilis*.

### *Hippea* Medium (DSMZ Medium 854)

**Composition** per 1010mL:

NaCl .....	25.0g
Sulfur, powdered .....	10.0g
Na-acetate .....	5.0g
MOPS [3-( <i>N</i> -morpholino) propane sulfonic acid] .....	3.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
Yeast extract .....	0.1g
Resazurin .....	0.5mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 6.1 ± 0.2 at 25°C

### **Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, sulfur, and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0. Sparge the medium with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture for 30 min. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Re-adjust the pH to 6.0–6.2. Dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture into anaerobe tubes or bottles containing 100.0mg sulfur powder per 10mL medium. Autoclave 20 min at 110°C. Prior to use inject 0.1mL sterile vitamin solution per 10.0mL medium.

**Use:** For the cultivation of *Hippea maritima*.

### *Hirschia* Medium

**Composition** per liter:

Pancreatic digest of casein .....	5.0g
HEPES .....	4.0g
Yeast extract .....	2.0g
Artificial seawater .....	250.0mL
Glucose solution .....	100.0mL

pH 7.4 ± 0.2 at 25°C

### **Glucose Solution:**

**Composition** per 100.0mL:

Glucose .....	0.25g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

### **Artificial Seawater:**

**Composition** per liter:

NaCl .....	27.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.38g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.78g
KCl .....	0.72g
NaHCO <sub>3</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.4g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Hirschia baltica*.

### *Histoplasma capsulatum* Agar

**Composition** per liter:

Agar .....	12.5g
Glucose .....	10.0g
Citric acid .....	10.0g
Potato starch .....	2.0g
α-Ketoglutaric acid .....	1.0g
L-Cystine-HCl·H <sub>2</sub> O .....	1.0g
Glutathione, reduced .....	0.5g
L-Asparagine .....	0.1g
L-Tryptophan .....	0.02g
Solution 1 .....	250.0mL
Solution 3 .....	40.0mL
Solution 2 .....	10.0mL
Solution 4 .....	10.0mL
Solution 8 .....	10.0mL
Solution 5 .....	1.0mL
Solution 6 .....	0.1mL
Solution 7 .....	0.1mL

pH 6.5 ± 0.2 at 25°C



**Solution 1:****Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	8.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	8.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.86g
CaCl <sub>2</sub> , anhydrous.....	0.08g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Store at 5°C.

**Solution 2:****Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.7g
MnCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.8g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.15g
HCl, concentrated.....	1.0mL

**Preparation of Solution 2:** Add 1.0mL of concentrated HCl to 100.0mL of distilled water in a 1.0L volumetric flask. Dissolve each component completely in the sequence given. Bring volume to 1.0L with distilled/deionized water. Store at 5°C. Discard if red color or red precipitate appears.

**Solution 3:****Composition** per 100.0mL:

Casein, acid-hydrolyzed, vitamin-free.....	10.0g
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**Preparation of Solution 3:** Add casein to distilled/deionized water and bring volume to 100.0mL.

**Solution 4:****Composition** per liter:

Calcium pantothenate.....	0.2g
Inositol.....	0.2g
Riboflavin.....	0.2g
Thiamine-HCl.....	0.2g
Nicotinamide.....	0.1g
Biotin.....	0.01g

**Preparation of Solution 4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Store at -20°C.

**Solution 5:****Composition** per 100.0mL:

Hemin.....	0.2g
NH <sub>4</sub> OH, concentrated.....	0.3mL

**Preparation of Solution 5:** Add hemin to approximately 30.0mL of distilled/deionized water. Add NH<sub>4</sub>OH. Mix thoroughly until dissolved. Bring volume to 100.0mL with distilled/deionized water. Store at 5°C.

**Solution 6:****Composition** per 10.0mL:

DL-Thioctic acid.....	0.01g
Ethanol (95% solution).....	10.0mL

**Preparation of Solution 6:** Add DL-thioctic acid to 10.0mL of ethanol. Mix thoroughly. Store at -20°C.

**Solution 7:****Composition** per 10.0mL:

Coenzyme A.....	0.01g
Na <sub>2</sub> S·5H <sub>2</sub> O (0.05% solution).....	0.2mL

**Preparation of Solution 7:** Prepare Na<sub>2</sub>S·5H<sub>2</sub>O solution in freshly boiled distilled/deionized water. Add coenzyme A to 9.8mL of distilled/deionized water. Mix thoroughly. Add freshly prepared Na<sub>2</sub>S·5H<sub>2</sub>O solution. Mix thoroughly. Store the solution at -20°C.

**Solution 8:****Composition** per 100.0mL:

Oleic acid.....	0.1g
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**Preparation of Solution 8:** Add oleic acid to 50.0mL of distilled/deionized water. Adjust pH to 9.0 with NaOH. Gently heat until dissolved. Bring volume to 100.0mL with distilled/deionized water. Store at 5°C.

**Preparation of Medium:** Add components—except agar, potato starch, and solution 8—to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 6.5 with 20% KOH solution. Filter sterilize. In a separate flask, add potato starch to 50.0mL of distilled/deionized water. Add the starch solution to 450.0mL of boiling distilled/deionized water. Add 10.0mL of solution 8 and the agar. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 70°C. Aseptically combine the two sterile solutions. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Histoplasma capsulatum* in the yeast phase. For the cultivation of *Histoplasma duboisii*, *Blastomyces dermatitidis*, and *Sporotrichum schenckii*.

**HP 6 Agar****Composition** per liter:

Agar.....	15.0g
Sodium glutamate.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
Yeast extract.....	1.0g
Cyanocobalamin.....	0.5mg
Glucose solution.....	100.0mL

pH 7.2 ± 0.2 at 25°C

**Glucose Solution:****Composition** per 100.0mL:

D-Glucose.....	5.0g
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**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 45°-50°C. Aseptically add sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

**HP 74 Broth****Composition** per liter:

Sodium glutamate.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
Yeast extract.....	2.0g
Glucose solution.....	100.0mL
Phosphate buffer solution.....	20.0mL

pH 6.5 ± 0.2 at 25°C

**Glucose Solution:****Composition** per 100.0mL:

D-Glucose.....	10.0g
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**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL.

Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Phosphate Buffer Solution:

**Composition** per 100.0mL:

K<sub>2</sub>HPO<sub>4</sub> ..... 6.81g

**Preparation of Phosphate Buffer Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except glucose solution and phosphate buffer solution, to distilled/deionized water and bring volume to 880.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile glucose solution and 20.0mL of sterile phosphate buffer solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

#### HP 101 Halophile Medium

**Composition** per liter:

NaCl ..... 100.0g  
Agar ..... 20.0g  
Peptone ..... 10.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 4.3g  
NaNO<sub>3</sub> ..... 2.0g  
Yeast extract ..... 1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

#### HP Medium

**Composition** per liter:

Pancreatic digest of soybean meal ..... 20.0g  
Beef extract ..... 10.0g  
Yeast extract ..... 6.0g  
Ammonium citrate ..... 5.0g  
Tween 80 ..... 0.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
MnSO<sub>4</sub>·4H<sub>2</sub>O ..... 0.05g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.04g  
Glucose solution ..... 10.0mL  
Tetracycline solution ..... 10.0mL

#### Glucose Solution:

**Composition** per 100.0mL:

Glucose ..... 10.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Tetracycline Solution:

**Composition** per 100.0mL:

Tetracycline ..... 10.0g

**Preparation of Tetracycline Solution:** Add tetracycline to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and tetracycline solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution and tetracycline solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and enumeration of *Leuconostoc* species.

#### HPC Agar (Heterotrophic Plate Count Agar) (m-HPC Agar)

**Composition** per liter:

Gelatin ..... 25.0g  
Pancreatic digest of gelatin ..... 20.0g  
Agar ..... 15.0g  
Glycerol ..... 10.0mL

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except glycerol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Add glycerol. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and enumeration of microorganisms from potable water sources, swimming pools, and other water specimens by the membrane filter method and heterotrophic plate count technique.

#### Hugh-Leifson's Glucose Broth

**Composition** per liter:

NaCl ..... 30.0g  
Glucose ..... 10.0g  
Agar ..... 3.0g  
Peptone ..... 2.0g  
Yeast extract ..... 0.5g  
Bromocresol Purple ..... 0.015g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Adjust pH to 7.4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria based on their ability to ferment glucose. Bacteria that ferment glucose turn the medium yellow.

#### Hungate's Habitat-Simulating Medium

**Composition** per 1140.2mL:

Rumen fluid ..... 333.0mL  
Mineral solution A ..... 167.0mL  
Mineral solution B ..... 167.0mL  
NaHCO<sub>3</sub> solution ..... 53.0mL  
L-Cysteine·HCl solution ..... 10.6mL  
Substrate solution ..... 10.6mL  
Resazurin solution ..... 1.0mL

**Mineral Solution A:****Composition per liter:**

NaCl .....	6.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
CaCl <sub>2</sub> .....	0.6g
MgSO <sub>4</sub> .....	0.6g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Mineral Solution B:**

K <sub>2</sub> HPO <sub>4</sub> .....	3.0
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**Preparation of Solution B:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Resazurin Solution:****Composition per 100.0mL:**

Resazurin .....	0.1g
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 100.0L. Mix thoroughly.

**L-Cysteine-HCl Solution:****Composition per 100.0mL:**

L-Cysteine-HCl .....	3.0g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to O<sub>2</sub>-free distilled/deionized water and bring volume to 100.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 2 min. Cool to 25°C under 100% N<sub>2</sub>. Seal tube with a stopper that is wired in place. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to O<sub>2</sub>-free distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Gas with 100% CO<sub>2</sub> for 15 min.

**Substrate Solution:****Composition per 100.0mL:**

Sugar .....	10.0g
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**Preparation of Substrate Solution:** Add sugar to O<sub>2</sub>-free distilled/deionized water. Mix thoroughly. Gas with 100% N<sub>2</sub> for 15 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add 167.0mL of solution A, 167.0mL of solution B, and 1.0mL of resazurin solution to distilled/deionized water and bring volume to 733.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Bring volume back to 733.0mL (some evaporation will have occurred) with O<sub>2</sub>-free distilled/deionized water. Cool to 45°–50°C under O<sub>2</sub>-free 100% CO<sub>2</sub>. Anaerobically add rumen fluid. Anaerobically distribute into tubes in 10.0mL volumes. Cap with butyl rubber stoppers. Place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Immediately prior to inoculation, aseptically and anaerobically add 0.1mL of sterile L-cysteine-HCl solution, 0.5mL of sterile NaHCO<sub>3</sub> solution, and 0.1mL of substrate solution per 10.0mL of medium in each tube.

**Use:** For the cultivation of *Bacteroides* species from rumens.

**HY Agar for *Flavobacterium*****Composition per liter:**

Agar .....	8.0g
Glutamic acid .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Glutamic acid may be replaced by 1.0g of folic acid if desired. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Flavobacterium* species.

**Hydrogen-Oxidizing Bacteria Medium****Composition per 1020.0mL:**

Solution I .....	1.0L
Solution II .....	10.0mL
Solution III .....	10.0mL

**Solution I:****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution .....	1.0mL

**Preparation of Solution I:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Solution II:****Composition per 100.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Ferric ammonium citrate .....	0.05g

**Preparation of Solution II:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution III:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution III:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 1.0L of cooled, sterile solution I, 10.0mL of cooled, sterile solution II, and 10.0mL of sterile solution III. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of hydrogen-oxidizing bacteria.

**Hydrogenobacter acidophilus Medium**  
(DSMZ Medium 743)**Composition per liter:**

Sulfur .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
CaCl <sub>2</sub> .....	1.0mg
NiSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.06mg
Trace elements solution .....	0.5mL

pH 3.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	28.0mg
MoO <sub>3</sub> .....	4.0mg
H <sub>3</sub> BO <sub>3</sub> .....	4.0mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	4.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	2.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Autoclave sulfur for 15 min at 9 psi pressure–113°C. Add components, except sulfur to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Add 5.0g sterile sulfur. Mix thoroughly by swirling. Adjust pH to 3.0 with HCl. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Hydrogenobaculum acidophilum*=*Hydrogenobacter acidophilus*.

**Hydrogenobacter halophilus Medium**  
(DSMZ Medium 744)**Composition per liter:**

NaCl .....	29.3g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	10.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
NiSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.6mg
Trace elements solution .....	0.5mL

pH 6.9 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	28.0mg
MoO <sub>3</sub> .....	4.0mg
H <sub>3</sub> BO <sub>3</sub> .....	4.0mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	4.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	2.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 6.9. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Hydrogenovibrio marinus*.

**Hydrogenobacter thermophilus Medium****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	4.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	10.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
NiSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.06mg
Trace elements solution .....	2.0mL

**Trace Elements Solution:****Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0mg
MoO <sub>3</sub> .....	1.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	1.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Calderobacterium hydrogenophilum* and *Hydrogenobacter thermophilus*.

**Hydrogenothermus hirschii Medium**  
(DSMZ Medium 783)**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.5g
NaHCO <sub>3</sub> .....	2.0g
KCl .....	0.65g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
Sulfur, powdered .....	0.5g
NH <sub>4</sub> Cl .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.15g
NaBr .....	0.1g
Trace elements solution .....	10.0mL
CaCO <sub>3</sub> solution .....	5.0mL

pH 7.0 ± 0.2 at 25°C

**CaCO<sub>3</sub> Solution:****Composition per 10mL:**

CaCO <sub>3</sub> .....	1.0g
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**Preparation of CaCO<sub>3</sub> Solution:** Add CaCO<sub>3</sub> to 10.0mL of distilled/deionized water. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritoltriactic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare the medium aerobically. Add sulfur to 900.0mL distilled/deionized water. Dissolve sulfur using Ultra-Turrax-dispersing instrument. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Adjust pH to 7.0 using H<sub>2</sub>SO<sub>4</sub>. Add 20.0mL medium into 100mL serum bottles. Seal with a rubber stopper. Change atmosphere to 80% H<sub>2</sub> + 20% CO<sub>2</sub> with an overpressure of 2 atmospheres. Autoclave for 20 min at 15 psi pressure–121°C. Cool to room temperature. Inject 20.0mL filter sterilized air and 0.1mL sterile CaCO<sub>3</sub> solution. Shake to mix.

**Use:** For the cultivation of *Hydrogenophilus hirschii* (*Hydrogenothermophilus hirschii*).

### Hydroxybenzoate Agar (*p*-Hydroxybenzoate Agar)

**Composition per liter:**

Agar .....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	3.0g
<i>p</i> -hydroxybenzoic acid .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
NaCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *p*-hydroxybenzoate-utilizing bacteria.

### *Hyperthermus butylicus* Medium

**Composition per 1010.0mL:**

NaCl .....	17.0g
Pancreatic digest of casein .....	6.0g
Sulfur, powdered .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.75g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0g
Yeast extract .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.75g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NH <sub>4</sub> Cl .....	0.5g
KCl .....	0.325g
NaBr .....	0.05g
H <sub>3</sub> BO <sub>3</sub> .....	0.015g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	7.5mg
Citric acid .....	5.0mg
KI .....	2.5mg
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 6.0–6.5 at 25°C

### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0 with KOH. Add distilled/deionized water to 1.0L.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0–6.5 with 6N H<sub>2</sub>SO<sub>4</sub>. Sparge with 100% N<sub>2</sub>. Sterilize by bringing to 90°C for 60 min on 3 consecutive days. Immediately prior to inoculation, add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Hyperthermus butylicus*.

### *Hyphomicrobium* Enrichment Medium

**Composition per 100.0mL:**

KNO <sub>3</sub> .....	0.04g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.48mg
FeCl <sub>3</sub> ·7H <sub>2</sub> O .....	0.02mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.01mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and enrichment of *Hyphomicrobium* species.

### *Hyphomicrobium* Medium

**Composition per liter:**

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	2.13g
KH <sub>2</sub> PO <sub>4</sub> .....	1.36g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	9.95mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	2.5mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.5mg

Urea solution.....	30.0mL
Methanol.....	4.0mL

**Urea Solution:****Composition per 100.0mL:**

Urea.....	20.0g
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**Preparation of Urea Solution:** Add urea to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Filter sterilize methanol. Add components, except urea solution and methanol, to distilled/deionized water and bring volume to 966.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile urea solution and sterile methanol. Mix thoroughly. Aseptically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Hyphomicrobium* species.

***Hyphomicrobium methylovorum* Medium****Composition per liter:**

Agar .....	15.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	3.0g
NaCl.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	5.0mg
Tap water.....	1.0L
Methanol.....	10.0mL
Vitamin mixture .....	5.0mL

**Vitamin Mixture:****Composition per liter:**

Inositol .....	200.0mg
Choline.....	100.0mg
Calcium DL-pantothenate.....	40.0mg
Niacin.....	40.0mg
Pyridoxine·HCl .....	40.0mg
Riboflavin.....	40.0mg
<i>p</i> -Aminobenzoic acid.....	20.0mg
Thiamine·HCl .....	20.0mg
Biotin .....	0.2mg
Folic acid.....	0.2mg
Cyanocobalamin .....	2.0μg

**Preparation of Vitamin Mixture:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Methanol:** Filter sterilize 10.0mL of methanol.

**Preparation of Medium:** Add components, except methanol and vitamin mixture, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile methanol and 5.0mL of sterile vitamin mixture. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Hyphomicrobium methylovorum*.

***Hyphomicrobium* Strain X Agar****Composition per liter:**

Agar .....	15.0g
Methylamine·HCl .....	3.4g
K <sub>2</sub> HPO <sub>4</sub> .....	1.55g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution.....	0.2mL
pH 7.2 ± 0.2 at 25°C	

**Trace Elements Solution:****Composition per liter:**

Disodium EDTA.....	50.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	22.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	5.54g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.61g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	1.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0 with KOH.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Hyphomicrobium* species.

***Hyphomonas* Enrichment Medium****Composition per liter:**

Peptone .....	0.05g
Yeast extract .....	0.05g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Hyphomonas* species.

***Hyphomonas* Medium****Composition per liter:**

Pancreatic digest of casein .....	2.0g
MgCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0g
Yeast extract .....	1.0g
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 using indicator paper. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Hyphomonas polymorpha*.

**IFO Agar****Composition per liter:**

Agar .....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
MnSO <sub>4</sub> ·4-6H <sub>2</sub> O.....	5.0mg
Riboflavin.....	0.02mg
Calcium pantothenate .....	0.02mg
Pyridoxine·HCl.....	0.02mg
Nicotinic acid .....	0.02mg
<i>p</i> -Aminobenzoic acid .....	0.01mg
Thiamine·HCl .....	0.01mg

Biotin .....	1.0μg
Methanol .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except agar and methanol, to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. In a separate flask, add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine the two sterile solutions. Aseptically add 10.0mL of filter-sterilized methanol. Mix thoroughly. Adjust pH to 7.0. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Hyphomicrobium methylovorum*.

### IFO Broth

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
MnSO <sub>4</sub> ·4-6H <sub>2</sub> O .....	5.0mg
Riboflavin .....	20.0μg
Calcium pantothenate .....	20.0μg
Pyridoxine-HCl .....	20.0μg
Nicotinic acid .....	20.0μg
<i>p</i> -Aminobenzoic acid .....	10.0μg
Thiamine-HCl .....	10.0μg
Biotin .....	1.0μg
Methanol .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of filter-sterilized methanol. Mix thoroughly. Adjust pH to 7.0. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Hyphomicrobium methylovorum*.

### IFO Medium 802

**Composition** per liter:

Polypeptone .....	10.0g
Yeast extract .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Sphingomonas asaccharolytica*, *Sphingomonas pruni*, *Sphingomonas mali*, and *Sphingomonas rosa*.

### *Ignicoccus* Medium (DSMZ Medium 897)

**Composition** per liter:

NaCl .....	13.65g
Sulfur, powdered .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.75g
Meat extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.38g
KCl .....	0.33g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.25g
NaBr .....	0.05g
H <sub>3</sub> BO <sub>3</sub> .....	15.0mg
SrCl <sub>3</sub> ·6H <sub>2</sub> O .....	7.50mg
KI .....	0.05mg
Resazurin .....	0.5mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
pH 5.5 ± 0.2 at 25°C	

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add 0.2g of Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution to distilled/deionized water and bring volume to 990.0mL. Sparge medium with N<sub>2</sub> gas for 30–60 min. Mix thoroughly. Add 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 5.5 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or bottles under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Heat the vessels containing medium in boiling water for 1 hr before inoculation. After inoculation pressurize the vessels with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.

**Use:** For the cultivation of *Ignicoccus islandicus* and *Ignicoccus pacificus*.

### Imhoff's Medium, Modified

**Composition** per liter:

NaCl .....	30.0g
NaHCO <sub>3</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Sodium acetate .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	0.7g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Sodium ascorbate .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Yeast extract .....	0.1g
Sodium sulfide solution .....	10.0mL
SLA trace elements solution .....	1.0mL
VA vitamin solution .....	1.0mL
pH 6.9–7.0 at 25°C	

### Sodium Sulfide Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.0g
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**Preparation of Sodium Sulfide Solution:** Add 2.0g of Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C under N<sub>2</sub>. Maintain under 100% N<sub>2</sub>.

### SLA Trace Elements Solution:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.07g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of SLA Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to pH 2–3. Filter sterilize.

#### VA Vitamin Solution:

**Composition per 500.0mL:**

Nicotinamide.....	0.17g
Thiamine-HCl .....	0.15g
<i>p</i> -Aminobenzoic acid.....	0.1g
Biotin .....	0.05g
Calcium pantothenate .....	0.05g
Pyridoxine-2HCl .....	0.05g
Cyanocobalamin .....	0.02g

**Preparation of VA Vitamin Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components—except sodium sulfide solution, SLA trace elements solution, and VA vitamin solution—to distilled/deionized water and bring volume to 988.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 1.0mL of sterile SLA trace elements solution and 1.0mL of sterile VA vitamin solution. Aseptically add 10.0mL of sterile sodium sulfide solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Rhodobacter adriaticus* and *Rhodobacter sulfidophilus*.

#### Imidazole Utilization Medium

**Composition per liter:**

Imidazole .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	3.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0mg
Molybdenum solution .....	1.0mL
Trace elements solution .....	1.0mL

pH 6.0 ± 0.2 at 25°C

#### Molybdenum Solution:

**Composition per 18.0mL:**

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5mg
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**Preparation of Molybdenum Solution:** Add components to distilled/deionized water and bring volume to 18.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution:

**Composition per 18.0mL:**

H <sub>3</sub> BO <sub>3</sub> .....	11.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	7.0mg
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·18 H <sub>2</sub> O .....	1.94mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
NiSO <sub>4</sub> ·6H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.62mg
KBr.....	0.5mg
KI.....	0.5mg
LiCl.....	0.5mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.5mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 18.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except molybdenum solution and trace elements solution, to distilled/deionized water and bring volume to 998.0mL. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min

at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 1.0mL of molybdenum solution and 1.0mL of trace elements solution. Mix thoroughly. Adjust pH to 6.0 with phosphoric acid. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudo-monas* species.

#### Inorganic Salts-Maltose Medium (DSMZ Medium 754)

**Composition per liter:**

Yeast extract .....	4.0g
Peptone .....	2.0g
Inorganic salt solution .....	980.0mL
Maltose solution .....	20.0mL

#### Maltose Solution:

**Composition per liter:**

Maltose .....	5.0g
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**Preparation of Maltose Solution:** Add maltose to 20.0mL distilled/deionized water. Mix thoroughly. Filter sterilize.

#### Inorganic Salt Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	49.37g
NaCl .....	43.8g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.29g

**Preparation of Inorganic Salt Solution:** Add components in the order CaCl<sub>2</sub>·2H<sub>2</sub>O, NaCl, MgSO<sub>4</sub>·7H<sub>2</sub>O to 900.0mL distilled/deionized water. After addition of each mix thoroughly to prevent precipitation. Add distilled/deionized water bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components except maltose solution to 980.0mL inorganic salt solution. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 20.0mL sterile maltose solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Spirochaeta halophila*.

#### Inositol Brilliant Green Bile Salts Agar (IBB Agar) (*Plesiomonas* Differential Agar)

**Composition per liter:**

Agar .....	15.0g
<i>meso</i> -Inositol .....	10.0g
Proteose peptone .....	10.0g
Bile salts No. 3 .....	8.5g
Meat extract.....	5.0g
NaCl .....	5.0g
Neutral Red (2% solution).....	1.25mL
Brilliant Green (0.1% solution).....	0.33mL

pH 7.2 ± 0.1 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation of *Aeromonas* and *Plesiomonas* species.

#### Iron Bacteria Isolation Medium

**Composition per liter:**

Agar.....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g



Glucose .....	0.15g
CaCO <sub>3</sub> .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
KCl .....	0.05g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g
Vitamin solution .....	10.0mL

**Vitamin Solution:****Composition per 10.0mL:**

Thiamine .....	0.4mg
Cyanocobalamin .....	0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation of iron bacteria.

**Iron-Oxidizing Medium****Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	300.0mL
H <sub>2</sub> SO <sub>4</sub> (10N) .....	1.0mL

pH 3.0–3.6 at 25°C

**FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:****Composition per 300.0mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	44.22g
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**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 44.22g of FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 300.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except FeSO<sub>4</sub>·7H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 700.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 300.0mL of sterile FeSO<sub>4</sub>·7H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the enumeration, isolation, and cultivation of iron and sulfur bacteria, such as *Thiobacillus ferrooxidans*.

**Iron Sulfite Agar****Composition per liter:**

Agar .....	12.0g
Pancreatic digest of casein .....	10.0g
Ferric citrate .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the detection of thermophilic anaerobic organisms.

**ISM Agar****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	49.2g
NaCl .....	43.5g
Agar .....	7.5g
Yeast extract .....	4.0g
Peptone .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.5g
Maltose solution .....	100.0mL

**Maltose Solution:****Composition per 100.0mL:**

Maltose .....	5.0g
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**Preparation of Maltose Solution:** Add maltose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except maltose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile maltose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Spirochaeta halophila*.

**Isoleucine Hydroxamate Medium****Composition per liter:**

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	7.0g
Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
L-Isoleucine hydroxamate .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Serratia marcescens*.

**Isonema Medium****Composition per liter:**

Pancreatic digest of casin .....	1.0g
Seawater .....	990.0mL
Horse serum, heat inactivated .....	10.0mL

**Preparation of Medium:** Add pancreatic digest of casein to seawater and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL of heat-inactivated horse serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Isonema papillatum*.

**Isosphaera Agar****Composition per 1000.5mL:**

Solution A .....	800.0mL
Solution B .....	100.0mL

Solution C .....	100.0mL
Vitamin solution .....	0.5mL
pH 7.6 ± 0.2 at 25°C	

**Solution A:**

**Composition per 800.0mL:**

Agar, noble .....	15.0g
NaCl .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.125g
KCl .....	0.125g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	80.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	75.0mg
FeCl <sub>3</sub> .....	73.0μg
Trace elements solution SL-7 .....	2.5mL

**Trace Elements Solution SL-7:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
H <sub>3</sub> BO <sub>3</sub> .....	62.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	17.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-7:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add agar to 400.0mL of distilled/deionized water. In another flask, add remaining components to 400.0mL of distilled/deionized water. Mix thoroughly. Adjust pH to 7.6 with NaOH. Remove any precipitate that forms by filtering through Whatman #1 filter paper. Autoclave both solutions separately for 15 min at 15 psi pressure–121°C. Aseptically combine the 2 sterile solutions. Cool to 50°–55°C.

**Solution B:**

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	0.42g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 50°–55°C.

**Solution C:**

**Composition per 100.5mL:**

Glucose .....	0.25g
Casamino acids .....	0.25g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 50°–55°C.

**Vitamin Solution:**

**Composition per 100.0mL:**

Nicotinic acid .....	200.0mg
Thiamine HCl .....	200.0mg
p-Aminobenzoic acid .....	20.0mg
Biotin .....	2.0mg
Vitamin B <sub>12</sub> .....	25.0μg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 50°–55°C.

**Preparation of Medium:** Aseptically combine 800.0mL of sterile solution A with 100.0mL of sterile solution B, 100.0mL of sterile solution C, and 0.5mL of sterile vitamin so-

lution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Isosphaera pallida*, a gliding, budding eubacterium from hot springs.

***Isosphaera pallida* Medium  
(DSMZ Medium 765)**

**Composition per 1005mL:**

Solution A .....	900.0mL
NaHCO <sub>3</sub> solution .....	100.0mL
Vitamin solution .....	5.0mL
pH 7.9 ± 0.2 at 25°C	

**Solution A:**

**Composition per 900mL:**

KCl .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
Glucose .....	0.25g
Casamino acids .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> .....	0.292mg
Trace elements solution SL-7a .....	1.0mL

**Trace Elements Solution SL-7a:**

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	200.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
H <sub>3</sub> BO <sub>3</sub> .....	60.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	40.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	20.0mg
HCl (25%) .....	1.0mL

**Preparation of Trace Elements Solution SL-7a:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with 95% air + 5% CO<sub>2</sub>.

**Vitamin Solution:**

**Composition per 100mL:**

Nicotinic acid .....	20.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	10.0mg
p-Aminobenzoic acid .....	0.2mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**NaHCO<sub>3</sub> Solution:**

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	4.2g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize. Seal in serum bottle under 100% CO<sub>2</sub>.

**Preparation of Medium:** Dispense under 95% air + 5% CO<sub>2</sub>. Fill serum bottles so that the gas-to-liquid ratio is about 5:1 (v/v). Sparge with 95% air + 5% CO<sub>2</sub>. Seal bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Using a syringe, inject 10.0mL sterile NaHCO<sub>3</sub> solution/90.0mL solution A. Then inject 0.5 mL sterile vitamin solution per 100.0mL of the resulting medium.

**Use:** For the cultivation of *Isosphaera pallida*=*Isocystis pallida*, a gliding, budding eubacterium from hot springs.

### JB Medium with Glucose

#### Composition per liter:

Yeast extract .....	15.0g
Pancreatic digest of casein .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.0g
Glucose .....	2.0g

pH 7.3–7.5 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus popilliae*.

### Johnson's Marine Medium

#### Composition per liter:

Peptone .....	5.0g
Yeast extract .....	1.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	0.3g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Filtered, aged seawater .....	750.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of marine bacteria.

### K101 *Flexibacter* Medium

#### Composition per liter:

Agar .....	10.0g
Casamino acids .....	1.0g
Glucose .....	1.0g
Tris(hydroxymethyl)aminomethane buffer .....	1.0g
CaCl <sub>2</sub> .....	0.1g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Sodium glycerophosphate .....	0.1g
Thiamine-HCl .....	1.0mg
Cyanocobalamin .....	1.0μg
Trace elements solution HO-LE .....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Trace Elements Solution HO-LE:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	2.85g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
Sodium tartrate .....	1.77g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.36g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.027g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
ZnCl <sub>2</sub> .....	0.02g

**Preparation of Trace Elements Solution HO-LE:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except trace elements solution HO-LE, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 1.0mL of trace elements solution HO-LE. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Cytophaga* species, *Flexibacter* species, *Herpetosiphon geysericola*, and *Myxococcus fulvus*.

### Keister's Modified TYI-S-33 Medium

#### Composition per liter:

Pancreatic digest of casein .....	20.0g
Glucose .....	10.0g
Yeast extract .....	10.0g
L-Cysteine-HCl .....	2.0g
NaCl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Bovine bile .....	0.75g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
Ascorbic acid .....	0.2g
Ferric ammonium citrate .....	22.8mg
Bovine serum, heat inactivated .....	100.0mL

**Preparation of Medium:** Add components, except bovine serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0L of sterile, heat-inactivated bovine serum. Mix thoroughly. Aseptically distribute into sterile, screw-capped tubes or flasks.

**Use:** For the cultivation of *Giardia cati*, *Giardia intestinalis*, and *Hexamita* species.

### Kerosene Mineral Salts Medium

#### Composition per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.02g
FeCl <sub>3</sub> .....	0.05g
Kerosene .....	20.0mL

pH 6.9–7.0 at 25°C

**Preparation of Medium:** Add components, except kerosene, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.9–7.0 with dilute NaOH. Distribute into tubes in 10.0mL volumes or flasks in 100.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Overlay tubes with 0.2mL of kerosene per tube. Overlay flasks with 2.0mL of kerosene per flask.

**Use:** For the cultivation of *Pseudomonas aeruginosa*.

### Ketolactonate Broth

#### Composition per liter:

Agar .....	20.0g
Lactose .....	10.0g
Yeast extract .....	10.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For use in the identification of agrobacteria and other bacteria based upon production of 3-ketogluconate. After incubation, Benedicts solution is added to the plates. Yellow zones around colonies indicate positive production of 3-ketogluconate.

### KF *Streptococcus* Agar

#### Composition per liter:

Agar .....	20.0g
Maltose .....	20.0g

Proteose peptone .....	10.0g
Sodium glycerophosphate .....	10.0g
Yeast extract .....	10.0g
NaCl .....	5.5g
Lactose .....	1.0g
NaN <sub>3</sub> .....	0.4g
Bromcresol Purple .....	0.015g
2,3,5-Triphenyltetrazolium chloride solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Caution:** Sodium azide is toxic. Azides also react with metals and disposal must be highly diluted.

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

### 2,3,5-Triphenyltetrazolium Chloride Solution:

Composition per 10.0mL:	
2,3,5-Triphenyltetrazolium chloride .....	0.1g

**Preparation of 2,3,5-Triphenyltetrazolium Chloride Solution:** Add 2,3,5-triphenyltetrazolium chloride to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except 2,3,5-triphenyltetrazolium chloride solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 2,3,5-triphenyltetrazolium chloride solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and enumeration of enterococci.

### Kidney Bean Agar

#### Composition per liter:

Kidney beans, dry .....	30.0g
Agar .....	15.0g

**Preparation of Medium:** Add dry kidney beans to distilled/deionized water and bring volume to 1.0L. Autoclave for 30min at 15 psi pressure–121°C. Filter solids through cheesecloth. Add agar to filtrate. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Conidiobolus stromioideus*, *Phialophora gregata*, *Phytophthora cactorum*, *Phytophthora cryptogea*, *Phytophthora erythroseptica*, *Phytophthora fragariae*, *Phytophthora heveae*, and *Phytophthora syringae*.

### Kievskaya Agar (Medium K)

#### Composition per liter:

Agar .....	15.0g
KNO <sub>3</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
MgSO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> .....	0.02g
FeCl <sub>3</sub> .....	1.0mg
pH 6.8–7.0 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 30 min at 3 psi pressure–105°C. Pour into sterile Petri dishes or leave in tubes. After inoculation, incubate in an atmosphere of natural gas.

**Use:** For the cultivation of *Rhodococcus luteus*, *Rhodococcus rhodochrous*, *Rhodococcus ruber*, *Rhodococcus* species, and other bacteria that can grow on natural gas.

### Kievskaya Broth with *n*-Hexadecane

#### Composition per liter:

KNO <sub>3</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
MgSO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> .....	0.02g
FeCl <sub>3</sub> .....	1.0mg
<i>n</i> -Hexadecane .....	10.0mL
pH 6.8–7.0 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Gordona terrae*, *Rhodococcus erythropolis*, *Rhodococcus luteus*, and *Rhodococcus maris*.

### King's Medium A

#### Composition per liter:

Proteose peptone .....	20.0g
Agar .....	15.0g
Glycerol .....	10.0g
K <sub>2</sub> SO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.5g
pH 7.2–7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the nonselective isolation, cultivation, and pigment production of *Pseudomonas*.

### King's Medium B

#### Composition per liter:

Agar .....	20.0g
Proteose peptone No. 3 .....	20.0g
K <sub>2</sub> HPO <sub>4</sub> , anhydrous .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g
Glycerol .....	15.0mL
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the nonselective isolation, cultivation, and pigment production of *Pseudomonas* species.

### Kleb Agar (m-Kleb Agar)

#### Composition per liter:

Agar .....	15.0g
Proteose peptone No. 3 .....	10.0g
NaCl .....	5.0g
Adonitol .....	5.0g

Beef extract .....	1.0g
Aniline Blue .....	0.1g
Sodium lauryl sulfate .....	0.1g
Phenol Red .....	0.025g
Ethanol (95% solution) .....	20.0mL
Carbenicillin solution .....	10.0mL
pH 7.4 ± 0.2 at 25°C	

**Carbenicillin Solution:****Composition per 10.0mL:**

Carbenicillin .....	0.05g
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**Preparation of Carbenicillin Solution:** Add carbenicillin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ethanol and carbenicillin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 20.0mL of ethanol and 10.0mL of carbenicillin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the enumeration of bacteria from waters.

***Klebsiella* Medium  
(m-*Klebsiella* Medium)**

**Composition per 1041.0mL:**

Agar .....	15.0g
Adonitol .....	4.0g
2× Salt solution .....	500.0mL
Uric acid solution .....	200.0mL
Phenol Red solution .....	10.0mL
Sodium taurocholate solution .....	30.0mL
Carbenicillin solution .....	1.0mL

**2× Salt Solution:****Composition per liter:**

KCl .....	8.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.0g
NaCl .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g

**Preparation of 2× Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Uric Acid Solution:****Composition per 200.0mL:**

Uric acid .....	0.3g
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**Preparation of Uric Acid Solution:** Dissolve uric acid in a small volume of 1N NaOH. Bring volume to 200.0mL with distilled/deionized water. Adjust pH to 7.1 with 1N HCl. Filter sterilize.

**Phenol Red Solution:****Composition per 10.0mL:**

Phenol Red .....	0.1g
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**Preparation of Phenol Red Solution:** Add Phenol Red to sterile distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Sodium Taurocholate Solution:****Composition per 30.0mL:**

Sodium taurocholate .....	0.4g
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**Preparation of Sodium Taurocholate Solution:** Add sodium taurocholate to sterile distilled/deionized water and bring volume to 30.0mL. Mix thoroughly.

**Carbenicillin Solution:****Composition per 1.0mL:**

Carbenicillin .....	5.0mg
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**Preparation of Carbenicillin Solution:** Add carbenicillin to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add adonitol and agar to 500.0mL of 2× salt solution. Bring volume to 800.0mL with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 200.0mL of uric acid solution, 30.0mL of sodium taurocholate solution, 10.0mL of Phenol Red solution, and 1.0mL of carbenicillin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the enumeration of *Klebsiella* species by the membrane filter method.

**Kligler Iron Agar****Composition per liter:**

Peptone .....	20.0g
Agar .....	12.0g
Lactose .....	10.0g
NaCl .....	5.0g
Beef extract .....	3.0g
Yeast extract .....	3.0g
Glucose .....	1.0g
Ferric citrate .....	0.3g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	0.3g
Phenol Red .....	0.05g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the differentiation and identification of Enterobacteriaceae based upon sugar fermentation and hydrogen sulfide production. Sugar fermentation is indicated by the medium turning yellow. H<sub>2</sub>S production results in the medium turning black.

**KoKo Medium****Composition per 1020.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	1.6g
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0g
Peptone, meat .....	1.0g
Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.16g
Resazurin .....	0.5mg
Glucose solution .....	100.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution .....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-4 .....	10.0mL
Wolfe's vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Glucose Solution:****Composition per 100.0mL:**

D-Glucose .....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

**Composition** per 10.0mL:

NaHCO<sub>3</sub> ..... 1.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### CaCl<sub>2</sub> Solution:

**Composition** per 10.0mL:

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.06g

**Preparation of CaCl<sub>2</sub> Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### L-Cysteine·HCl·H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

L-Cysteine·HCl·H<sub>2</sub>O ..... 0.3g

**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### Trace Elements Solution SL-4:

**Composition** per liter:

EDTA ..... 0.5g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

Trace elements solution SL-6 ..... 100.0mL

#### Trace Elements Solution SL-6:

**Composition** per liter:

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.5g

H<sub>3</sub>BO<sub>3</sub> ..... 0.3g

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.03g

NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02g

CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine·HCl ..... 10.0mg

p-Aminobenzoic acid ..... 5.0mg

Lipoic acid ..... 5.0mg

Nicotinic acid ..... 5.0mg

Riboflavin ..... 5.0mg

Thiamine·HCl ..... 5.0mg

Calcium DL-pantothenate ..... 5.0mg

Biotin ..... 2.0mg

Folic acid ..... 2.0mg

Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except glucose solution, NaHCO<sub>3</sub> solution, CaCl<sub>2</sub>·2H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, L-cysteine·HCl·H<sub>2</sub>O solution, and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 850.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 100.0mL of sterile glucose solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution, 10.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. The pH should be 7.0. A buffer solution of 1% MOPS from a 10% anaerobic solution at pH 6.9–7.0 may be added aseptically and anaerobically to enhance the buffer capacity of the medium. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermoanaerobacter italicus*.

#### Korthof Medium

**Composition** per 1088.0mL:

NaCl ..... 1.4g

Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O ..... 0.88g

Peptone ..... 0.8g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.24g

CaCl<sub>2</sub> ..... 0.04g

KCl ..... 0.04g

NaHCO<sub>3</sub> ..... 0.02g

Rabbit serum, inactivated ..... 80.0mL

Rabbit hemoglobin solution ..... 8.0mL

pH 7.2 ± 0.2 at 25°C

#### Rabbit Hemoglobin Solution:

**Composition** per 20.0mL:

Rabbit blood clot ..... 10.0mL

**Preparation of Rabbit Hemoglobin Solution:** Add rabbit blood clot to 10.0mL of distilled/deionized water. Lyse the clot by freezing and thawing.

**Preparation of Medium:** Add components, except rabbit serum and rabbit hemoglobin solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Cool to 25°C. Filter through Whatman #1 filter paper. Distribute into flasks in 100.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 8.0mL of rabbit serum and 0.8mL of rabbit hemoglobin solution to each flask. Mix thoroughly.

**Use:** For the cultivation of *Leptospira* species.

#### Koser Citrate Medium

**Composition** per liter:

Sodium citrate ..... 3.0g

NaNH<sub>4</sub>HPO<sub>4</sub>·4H<sub>2</sub>O ..... 1.5g

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

pH 6.7 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the differentiation of *Escherichia coli* and *Enterobacter aerogenes* based on citrate utilization.

#### KYE Agar

**Composition per liter:**

Agar .....	15.0g
NaNO <sub>3</sub> .....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.15g
NaCl .....	0.1g
FeCl <sub>3</sub> .....	10.0mg

pH 10.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of alkaliphilic bacteria.

#### L and F Basal Salts, Modified with Heptadecane

**Composition per liter:**

NH <sub>4</sub> Cl .....	2.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.09g
KCl .....	0.04g
CaCl <sub>2</sub> .....	0.015g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.07mg
H <sub>3</sub> BO <sub>3</sub> .....	0.01mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01mg
MoO <sub>3</sub> .....	0.01mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	5.0μg
Heptadecane .....	2.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except heptadecane, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute equally into four 250.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C. Filter sterilize heptadecane. To one 250.0mL fraction of basal salts, aseptically add 0.5mL of sterile heptadecane. Pour mixture into a sterile blender. Homogenize slowly to mix heptadecane with basal salts and not to create excess bubbles. Rapidly distribute medium to sterile screw-capped tubes. Chill tubes quickly in an ice pack or in the refrigerator. Allow tubes to solidify in a slanted position.

**Use:** For the cultivation and maintenance of *Thermoleophilum album* and *Thermoleophilum minutum*

#### L Medium (ATCC Medium 167)

**Composition per liter:**

Agar .....	20.0g
NaNO <sub>3</sub> .....	2.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.09g

KCl .....	0.04g
CaCl <sub>2</sub> .....	0.015g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
Salts solution .....	1.0mL

#### Salts Solution:

**Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
MoO <sub>3</sub> .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg

**Preparation of Salts Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Methylococcus capsulatus* and *Pseudomonas methanica*.

#### L Medium with Methanol

**Composition per liter:**

Agar .....	20.0g
NaNO <sub>3</sub> .....	2.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.09g
KCl .....	0.04g
CaCl <sub>2</sub> .....	0.015g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
Methanol .....	20.0mL
Salts solution .....	1.0mL

#### Salts Solution:

**Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
MoO <sub>3</sub> .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg

**Preparation of Salts Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Filter sterilize methanol. Aseptically add 20.0mL of sterile methanol to cooled, sterile basal medium. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylobacillus glycogenes*.

#### Lab-Lemco Agar

**Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
Lab-Lemco meat extract .....	3.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a variety of heterotrophic microorganisms.

### Lactate Agar

**Composition per liter:**

Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.8g
Agar .....	2.0g
Peptone .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.52g
Sodium lactate (60% solution) .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Serpens flexibilis*.

### Lactose Broth

**Composition per liter:**

Lactose .....	5.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.9 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing an inverted Durham tube in 10.0mL volumes. Autoclave for 12 min at 15 psi pressure–121°C. Cool broth quickly to 25°C. For testing water samples with 10.0mL volumes, prepare medium double strength.

**Use:** For the detection of lactose-fermenting, Gram-negative coliforms, as a preenrichment broth for *Salmonella* species, and in the study of lactose fermentation of bacteria in general.

### Lactose Minimal Medium

**Composition per liter:**

Agar .....	20.0g
Lactose .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	5.0g
NH <sub>4</sub> Cl .....	2.0g
NaCl .....	1.0g
MgSO <sub>4</sub> .....	0.1g
Yeast extract .....	0.1g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas campestris*.

### Lactose Ricinoleate Broth

**Composition per liter:**

Lactose .....	10.0g
Peptone .....	5.0g
Sodium ricinoleate .....	1.0g
pH 7.6 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the selective cultivation of members of the Enterobacteriaceae.

### Lauryl Sulfate Broth (m-Lauryl Sulfate Broth)

**Composition per liter:**

Peptone .....	39.0g
Lactose .....	30.0g
Yeast extract .....	6.0g
Sodium lauryl sulfate .....	1.0g
Phenol Red .....	0.2g
pH 7.4 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into bottles or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and enumeration of coliform bacteria, especially *Escherichia coli*, in water by the membrane filter method.

### Lauryl Sulfate Broth (Lauryl Tryptose Broth)

**Composition per liter:**

Pancreatic digest of casein .....	20.0g
Lactose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.75g
KH <sub>2</sub> PO <sub>4</sub> .....	2.75g
Sodium lauryl sulfate .....	0.1g
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing an inverted Durham tube in 10.0mL volumes. Autoclave for 12 min at 15 psi pressure–121°C. Cool broth quickly to 25°C. For testing water samples with 10.0mL volumes, prepare medium double strength.

**Use:** For the detection of coliform bacteria in a variety of specimens. Also, for the enumeration of coliform bacteria by the multiple-tube fermentation technique.

### Lauryl Sulfate Broth with MUG

**Composition per liter:**

Pancreatic digest of casein .....	20.0g
Lactose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.75g
KH <sub>2</sub> PO <sub>4</sub> .....	2.75g
Sodium lauryl sulfate .....	0.1g
4-Methylumbelliferyl- β-D-glucuronide (MUG) .....	0.05g
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.



Distribute into tubes containing an inverted Durham tube in 10.0mL volumes. Autoclave for 12 min at 15 psi pressure–121°C. Cool broth quickly to 25°C. For testing water samples with 10.0mL volumes, prepare medium double strength.

**Use:** For the detection of *Escherichia coli* in water and by a fluorogenic procedure.

### **Lauryl Tryptose Mannitol Broth with Tryptophan**

**Composition per liter:**

Pancreatic digest of casein .....	20.0g
Lactose .....	5.0g
NaCl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.75g
KH <sub>2</sub> PO <sub>4</sub> .....	2.75g
Sodium lauryl sulfate .....	0.1g
L-Tryptophan .....	0.2g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes containing an inverted Durham tube in 10.0mL volumes. Autoclave for 10 min at 10 psi pressure–115°C. Cool broth quickly to 25°C.

**Use:** For the detection of *Escherichia coli* in water samples.

### **LAVMm2 Medium**

**Composition per liter:**

Lactalbumin hydrolysate .....	10.0g
Sodium acetate .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.3mg
Nitritoltri-acetic acid .....	19.1mg
CaCl <sub>2</sub> .....	11.1mg
FeSO <sub>4</sub> .....	0.152mg
Thiamine-HCl .....	0.05mg
Cupric acetate .....	0.04mg
Biotin .....	0.02mg

pH 8.0–8.1 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with Na<sub>2</sub>CO<sub>3</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. The pH should be 8.0–8.1 after autoclaving.

**Use:** For the cultivation of *Caryophanon latum*.

### **LB Agar**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	10.0g
NaCl .....	5.0g
Yeast extract .....	5.0g
1N NaOH .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 25 min at 15 psi pressure–121°C. Pour into sterile Petri dishes in 35–40.0mL volumes.

**Use:** For the cultivation of *Escherichia coli*.

### **Lead Acetate Agar**

**Composition per liter:**

Agar .....	15.0g
Peptone .....	15.0g
Proteose peptone .....	5.0g
Glucose .....	1.0g
Lead acetate .....	0.2g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	0.08g

pH 6.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Allow tubes to cool in a slanted position.

**Use:** For the cultivation and differentiation of Gram-negative coliform bacteria based on H<sub>2</sub>S production. Bacteria that produce H<sub>2</sub>S turn the medium brown.

### **Legionella Selective Agar**

**Composition per liter:**

Agar .....	15.0g
ACES (2-[(2-Amino-2-oxoethyl)-amino]ethane sulfonic acid) buffer .....	10.0g
Yeast extract .....	10.0g
Charcoal, activated .....	2.0g
α-Ketoglutarate .....	1.0g
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> solution .....	10.0mL
Antibiotic solution .....	10.0mL

pH 6.85–7.0 at 25°C

**Source:** This medium is available as a prepared medium from BD Diagnostic Systems.

### **L-Cysteine·HCl·H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.4g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

### **Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:**

**Composition per 10.0mL:**

Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> .....	0.25g
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**Preparation of Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> Solution:** Add Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

### **Antibiotic Solution:**

**Composition per 10.0mL:**

Anisomycin .....	10.0mg
Colistin .....	3.75mg
Vancomycin .....	2.0mg

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components—except L-cysteine·HCl·H<sub>2</sub>O, Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>, and antibiotic solutions—to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45–50°C. Aseptically add sterile L-cysteine·HCl·H<sub>2</sub>O, Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub>, and antibiotic solutions. Mix thoroughly. Pour into sterile Petri dishes. Swirl medium while pouring to keep charcoal in suspension.

**Use:** *Legionella* selective agar is used in qualitative procedures for the isolation of *Legionella* species.

### Legume Extract Agar

**Composition** per liter:

Alfalfa roots .....	35.0g
Agar .....	20.0g
Soybean meal .....	10.0g
Sucrose .....	10.0g
CaCO <sub>3</sub> .....	5.0g
Glucose .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.1g
NaCl .....	0.1g
FeCl <sub>3</sub> .....	1.0mg

**Preparation of Medium:** Wash the alfalfa roots well and cut them up. Add 10.0g of soybean meal. Add three times the volume of distilled/deionized water. Steam for 1 hr. Let stand at 25°C overnight. Bring volume to 1.0L with distilled/deionized water. Filter through paper pulp. To the filtrate, add the K<sub>2</sub>HPO<sub>4</sub>, CaCl<sub>2</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, NaCl, FeCl<sub>3</sub>, and agar. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 45°–50°C. Add the CaCO<sub>3</sub>, sucrose, and glucose. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 20 min at 10 psi pressure–115°C.

**Use:** For the cultivation of *Rhizobium* species.

### Leifson Medium

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	2.0g
MgCl <sub>2</sub> .....	1.0g
Yeast extract .....	1.0g

pH 8.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 8.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the direct isolation and routine culturing of *Hyphomonas* species.

### Leptospira Medium

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> Fe(SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	6.0g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.53g
L-Asparagine .....	0.5g
Glycerol .....	0.2g
Tween 60 .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.15g
KH <sub>2</sub> PO <sub>4</sub> .....	0.069g
Tween 80 .....	0.05g
EDTA .....	0.01g
CaCO <sub>3</sub> .....	4.0mg
Thiamine-HCl .....	1.0mg
Vitamin B <sub>12</sub> .....	1.0μg

pH 7.4–7.6 at 25°C

**Preparation of Medium:** Add components, except thiamine-HCl, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mg of thiamine-HCl. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leptospira* species.

### Leptospira Medium, EMJH

(*Leptospira* Medium,

Ellinghausen-McCullough/Johnson-Harris)

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
NH <sub>4</sub> Cl .....	0.25g
Thiamine .....	5.0mg
Rabbit serum .....	100.0mL

pH 7.5 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except rabbit serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add sterile rabbit serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Leptospira* species.

### Leptospira Medium, Modified

**Composition** per liter:

Agar .....	1.5g
NaCl .....	0.5g
Peptone .....	0.3g
Beef extract .....	0.2g
Hemin solution .....	2.5mL
Sterile rabbit serum .....	100.0mL

pH 7.3 ± 0.1 at 25°C

### Hemin Solution:

**Composition** per 100.0mL:

Hemin .....	0.05g
NaOH (1N solution) .....	1.0mL

**Preparation of Hemin Solution:** Add hemin to 1.0mL of 1N NaOH solution. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add components, except hemin solution and rabbit serum, to distilled/deionized water and bring volume to 897.5mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 2.5mL of sterile hemin solution and 100.0mL of sterile rabbit serum. Mix thoroughly. The pH of the medium should be 7.3. Store at 4°C for 24 hr. Inactivate medium at 56°C for 60 min. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Leptospira biflexa*, *Leptospira borgpetersenii*, *Leptospira interrogans*, *Leptospira meyeri*, *Leptospira noguchii*, *Leptospira santarosai*, and *Leptospira weili*.

### Leptospira Protein-Free Medium

(*Leptospira* PF Medium)

**Composition** per liter:

TES (N-Tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid) buffer .....	1.2g
NaCl .....	0.9g
Sodium pyruvate .....	0.2g
CT-Tween 60 .....	12.0mL
CT-Tween 40 .....	3.0mL
MgCl <sub>2</sub> -CaCl <sub>2</sub> solution .....	1.0mL
Cyanocobalamin (0.02% solution) .....	1.0mL

Glycerol (10% solution).....	1.0mL
KH <sub>2</sub> PO <sub>4</sub> (1% solution).....	1.0mL
MnSO <sub>4</sub> ·H <sub>2</sub> O (0.1% solution).....	1.0mL
ZnSO <sub>4</sub> (0.4% solution).....	0.1mL
pH 7.6 ± 0.2 at 25°C	

**CT-Tween 60:****Composition** per 200.0mL:

Charcoal, Norit A.....	40.0g
Tween 60.....	20.0g

**Preparation of CT-Tween 60:** Add Tween 60 to 200.0mL of distilled/deionized water. Mix thoroughly. While stirring, add charcoal. Stir mixture for 18 hr at 25°C. Allow charcoal to settle out of suspension for 18 hr at 4°C. Carefully decant the Tween solution off the sediment. Centrifuge the Tween solution at 10,000 × g for 1 hr. Decant supernatant solution. Pass Tween solution through a thin-channel ultrafiltration XM 100 membrane. Store stock solution at -20°C.

**CT-Tween 40:****Composition** per 200.0mL:

Charcoal, Norit A.....	40.0g
Tween 40.....	20.0g

**Preparation of CT-Tween 40:** Add Tween 40 to 200.0mL of distilled/deionized water. Mix thoroughly. While stirring, add charcoal. Stir mixture for 18 hr at 25°C. Allow charcoal to settle out of suspension for 18 hr at 4°C. Carefully decant the Tween solution off the sediment. Centrifuge the Tween solution at 10,000 × g for 1 hr. Decant supernatant solution. Pass Tween solution through a thin-channel ultrafiltration XM 100 membrane. Store stock solution at -20°C.

**MgCl<sub>2</sub>-CaCl<sub>2</sub> Solution:****Composition** per 100.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.5g

**Preparation of MgCl<sub>2</sub>-CaCl<sub>2</sub> Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leptospira* species.

***Leptospirillum ferrooxidans* Medium****Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	30.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.147g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.13g
KH <sub>2</sub> PO <sub>4</sub> .....	27.0mg
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	25.0mg
Trace elements solution.....	1.0mL
pH 2.0 ± 0.2 at 25°C	

**Trace Elements Solution:****Composition** per liter:

CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.12g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	85.2mg
ZnCl <sub>2</sub> .....	70.0mg
H <sub>3</sub> BO <sub>3</sub> .....	31.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add H<sub>2</sub>SO<sub>4</sub> to 900.0mL of distilled/deionized water and bring pH to 3.0. Add components. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Adjust pH to 2.0 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Leptospirillum ferrooxidans*.

***Leptospirillum* HH Medium  
(DSMZ Medium 882)****Composition** per 1001mL:

Solution A.....	950.0mL
Solution B.....	50.0mL
Trace elements solution.....	1.0mL
pH 1.8 ± 0.2 at 25°C	

**Solution A:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	147.0mg
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	132.0mg
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	53.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	27.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 1.8 with 10N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 30 min at 112°C. Cool to room temperature.

**Solution B:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	20.0g
H <sub>2</sub> SO <sub>4</sub> , 0.25N.....	50.0mL

**Preparation of Solution B:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to 50.0mL 0.25N H<sub>2</sub>SO<sub>4</sub>. Mix thoroughly. The pH should be 1.2. Autoclave for 30 min at 112°C. Cool to room temperature.

**Trace Elements Solution:**

ZnCl <sub>2</sub> .....	68.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	67.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	64.0mg
MnCl <sub>2</sub> ·2H <sub>2</sub> O.....	62.0mg
H <sub>3</sub> BO <sub>3</sub> .....	31.0mg
Na <sub>2</sub> MoO <sub>4</sub> .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 1.8 with 10N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure-121°C. Cool to room temperature.

**Preparation of Medium:** Aseptically mix 950.0mL of solution A and 50.0mL solution B. Mix thoroughly. Aseptically add 1.0mL trace elements solution. Mix thoroughly. Adjust pH to 1.8.

**Use:** For the cultivation of *Acidithiobacillus ferrooxidans*=*Thiobacillus ferrooxidans* and *Leptospirillum* spp.

***Leptothrix ochracea* Medium****Composition** per liter:

Agar.....	10.0g
Manganous acetate.....	0.1g
Manganese bicarbonate solution.....	100.0mL

**Manganese Bicarbonate Solution:****Composition** per 100.0mL:

MnCO <sub>3</sub> .....	2.0g
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**Preparation of Manganese Bicarbonate Solution:** Add MnCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gas with 100% CO<sub>2</sub> for 20 min. Filter through Whatman #1 filter paper.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Leptothrix ochracea*.

#### *Leptothrix* 2× PYG Medium

**Composition** per liter:

HEPES (N-2-Hydroxyethyl piperazine-N'-2-ethanesulfonic acid) buffer	3.57g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.6g
Glucose	0.5g
Peptone	0.5g
Yeast extract	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.07g
MnSO <sub>4</sub> ·H <sub>2</sub> O	0.017g
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Leptothrix discophora*.

#### *Leptothrix* Strains Medium

**Composition** per liter:

Agar	12.0g
Peptone	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Ferric ammonium citrate	0.15g
CaCl <sub>2</sub>	0.05g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O	0.01g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Leptothrix* species.

#### *Leuconostoc* Medium

**Composition** per liter:

CaCO <sub>3</sub>	50.0g
Malt extract	50.0g
Agar	15.0g
NaCl	2.5g
Beef extract	1.0g
Polypeptone	1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 10 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Leuconostoc mesenteroides*.

#### *Leuconostoc oenos* Medium

**Composition** per liter:

Glucose	10.0g
Peptone	10.0g
Yeast extract	5.0g
MnSO <sub>4</sub> ·4H <sub>2</sub> O	0.1g

Tomato juice	250.0mL
L-Cysteine-HCl solution	10.0mL
pH 4.8 ± 0.2 at 25°C	

#### L-Cysteine-HCl Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl	0.5g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine-HCl solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add sterile L-cysteine-HCl solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leuconostoc oenos*.

#### *Leucothrix* Medium

**Composition** per liter:

Pancreatic digest of casein	10.0g
Synthetic seawater	1000.0mL
pH 7.8 ± 0.2 at 25°C	

#### Synthetic Seawater:

**Composition** per liter:

NaCl	24.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	11.0g
Na <sub>2</sub> SO <sub>4</sub>	4.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O	2.0g
KCl	0.7g
KBr	0.1g
SrCl <sub>2</sub> ·6H <sub>2</sub> O	0.04g
H <sub>3</sub> BO <sub>3</sub>	0.03g
NaSiO <sub>3</sub> ·9H <sub>2</sub> O	5.0mg
NaF	3.0mg
NH <sub>4</sub> NO <sub>3</sub>	2.0mg
FePO <sub>4</sub> ·4H <sub>2</sub> O	1.0mg

**Preparation of Synthetic Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add 10.0g of pancreatic digest of casein to 1.0L of synthetic seawater. Mix thoroughly. Adjust pH to 7.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Leucothrix* species.

#### *Leucothrix mucor* Medium

**Composition** per liter:

NaCl	11.75g
Monosodium glutamate	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	5.35g
Na <sub>2</sub> SO <sub>4</sub>	2.0g
Sodium lactate	2.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O	1.12g
Tris (hydroxymethyl) amino methane	0.5g
KCl	0.35g
Na <sub>2</sub> HPO <sub>4</sub>	0.05g
pH 7.6 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Leucothrix mucor*.

**Levine EMB Agar**  
**(Levine Eosin Methylene Blue Agar)**  
**(Eosin Methylene Blue Agar, Levine)**  
**(LEMB Agar)**

**Composition per liter:**

Agar .....	15.0g
Lactose .....	10.0g
Peptone .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Eosin Y .....	0.4g
Methylene Blue .....	0.065mg

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and differentiation of Gram-negative enteric bacteria based on lactose fermentation. Bacteria that ferment lactose, especially the coliform bacterium *Escherichia coli*, appear as colonies with a green metallic sheen or blue-black to brown color. Bacteria that do not ferment lactose appear as colorless or transparent light purple colonies.

**LHET2 Medium****Composition per liter:**

Solution A .....	500.0mL
Solution B .....	500.0mL

pH 2.5–3.0 at 25°C

**Solution A:****Composition per 500.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.51g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
Pancreatic digest of casein .....	0.06g
NaCl .....	0.02g
Papaic digest of soybean meal .....	0.01g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 2.5–3.0 with 1N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution B:****Composition per 500.0mL:**

Agar .....	12.0g
Glucose .....	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine sterile solution A and sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Acidiphilium crypum*.

**LHET2 Medium with Yeast Extract**  
**or Yeast Autolysate**

**Composition per liter:**

Solution A .....	500.0mL
Solution B .....	500.0mL

pH 2.5–3.0 at 25°C

**Solution A:****Composition per 500.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.51g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
Yeast extract or yeast autolysate .....	0.1g
Pancreatic digest of casein .....	0.06g
NaCl .....	0.02g
Papaic digest of soybean meal .....	0.01g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 2.5–3.0 with 1N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution B:****Composition per 500.0mL:**

Agar .....	12.0g
Glucose .....	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine sterile solution A and sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Acidiphilium angustum*, *Aacidiphilium facilis*, and *Acidiphilium rubrum*.

**Lichen Fungi Medium****Composition per liter:**

Agar .....	20.0g
Malt extract .....	20.0g
Yeast extract .....	2.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of numerous fungi from lichen symbiotic relationships.

**Lima Bean Agar**  
**(ATCC Medium 322)**

**Composition per liter:**

Lima beans, infusion from 62.5g .....	8.0g
Agar .....	15.0g

pH 5.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of phytopathological fungi and other fungi.

***Limnobacter* Medium  
(DSMZ Medium 919)****Composition per liter:**

Yeast extract.....	0.5g
Proteose peptone.....	0.5g
Casamino acids.....	0.5g
Glucose.....	0.5g
Soluble starch.....	0.5g
Sodium pyruvate.....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Limnobacter thiooxidans*.

**Lipovitellin Salt Mannitol Agar****Composition per liter:**

NaCl.....	75.0g
Egg yolk.....	20.0g
Agar.....	15.0g
D-Mannitol.....	10.0g
Polypeptone.....	10.0g
Beef extract.....	1.0g
Phenol Red.....	0.025g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the detection of *Staphylococcus aureus* in swimming pool water based on lipovitellin-lipase activity and mannitol fermentation. *Staphylococcus aureus* and other bacteria with lipovitellin-lipase activity attack the egg yolk and appear as colonies surrounded by an opaque zone. Bacteria that ferment mannitol appear as colonies surrounded by a yellow zone.

***Lobosphaera* Medium****Composition per liter:**

Polypeptone.....	10.0g
Glycerol.....	7.5g
Yeast extract.....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Lobosphaera* species.

**Low Phosphate Buffered  
Basal Medium, Modified****Composition per 1030.0mL:**

Pectin.....	4.0g
NH <sub>4</sub> Cl.....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.72g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
Reducing agent.....	20.0mL
Yeast extract solution.....	10.0mL

Trace minerals.....	10.0mL
Vitamins.....	5.0mL
Resazurin (0.2% solution).....	1.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (2.5% solution).....	0.03mL

pH 7.3 ± 0.1 at 25°C

**Reducing Agent:****Composition per 20.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Reducing Agent:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Gas with 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 45 min at 15 psi pressure–121°C. Use freshly prepared solution.

**Yeast Extract Solution:****Composition per 10.0mL:**

Yeast extract.....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C. Cool to 25°C.

**Trace Minerals:****Composition per liter:**

Nitrilotriacetic acid.....	12.8g
NaCl.....	1.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.16g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
ZnCl <sub>2</sub> .....	0.1g
NiSO <sub>4</sub> ·6H <sub>2</sub> O.....	0.026g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Minerals:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	0.01g
Thiamine-HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
Calcium pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except yeast extract solution and reducing agent, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Cool under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Anaerobically distribute into tubes in 6.0mL volumes. Autoclave for 45 min at 15 psi pressure–121°C. Aseptically add 0.06mL of sterile yeast extract solution to each tube. Mix thoroughly. Immediately prior to inoculation, aseptically add 0.12mL of sterile reducing agent to each tube. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Clostridium thermosulfurogenes*.

#### LPBM Acido-Thermophile Medium

##### Composition per liter:

Agar .....	20.0g
Cellulose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
Cellobiose .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g

pH 5.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except cellulose and cellobiose, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.2 with H<sub>3</sub>PO<sub>4</sub>. Add cellulose and cellobiose. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Acidothermus cellulolyticus*.

#### LTH Medium for *Thiothrix*

##### Composition per liter:

HEPES (N-[2-Hydroxyethyl]piperazine- N'-2-ethanesulfonic acid) buffer.....	2.38.g
Sodium lactate .....	1.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
KH <sub>2</sub> PO <sub>4</sub> .....	85.0mg
EDTA .....	3.0mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.0mg
Wolfe's vitamin solution .....	10.0mL

pH 7.3 ± 0.2 at 25°C

#### Wolfe's Vitamin Solution:

##### Composition per liter:

Pyridoxine·HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Wolfe's vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 7.3 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiothrix* species.

#### Luminous Medium

##### Composition per liter:

NaCl .....	30.0g
Agar .....	20.0g
NH <sub>4</sub> Cl .....	5.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.1g
CaCO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
KCl .....	0.75g
Tris buffer (1M solution, pH 7.5) .....	50.0mL
Glycerol .....	3.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alteromonas hanedai*, *Photobacterium* species, *Shewanella hanedai*, and *Vibrio* species.

#### Lysine Decarboxylase Broth, Falkow

##### Composition per liter:

Peptone .....	5.0g
L-Lysine .....	5.0g
Yeast extract .....	3.0g
Glucose .....	1.0g
Bromocresol Purple .....	0.02g

pH 6.5–6.8 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.5–6.8. Distribute into tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria, especially *Salmonella*, based on their ability to decarboxylate lysine. Bacteria that decarboxylate lysine turn the medium turbid purple.

#### Lysine Decarboxylase Broth, Taylor Modification

##### Composition per liter:

L-Lysine .....	5.0g
Yeast extract .....	3.0g
Glucose .....	1.0g
Bromocresol Purple .....	0.02g

pH 6.1 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.1. Distribute into tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria, especially *Salmonella*, based on their ability to decarboxylate lysine. Bacteria that decarboxylate lysine turn the medium turbid purple.

**Lysine Decarboxylase Broth,  
Taylor Modification  
(Lysine Decarboxylase Broth)**

**Composition per liter:**

L-Lysine.....	5.0g
Peptone.....	5.0g
Yeast extract.....	3.0g
Glucose.....	1.0g
Bromcresol Purple.....	0.02g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.1. Distribute into tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria, especially *Salmonella*, based on their ability to decarboxylate lysine. Bacteria that decarboxylate lysine turn the medium turbid purple.

**Lysine Decarboxylase Medium**

**Composition per liter:**

Glucose.....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
L-Lysine-HCl.....	0.5g

pH 4.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 4.6. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically distribute into sterile tubes in 1.0mL volumes.

**Use:** For the cultivation and differentiation of Gram-negative, nonfermentative bacteria based on their ability to decarboxylate lysine. Bacteria that decarboxylate lysine turn the medium turbid purple.

**Lysine Iron Agar**

**Composition per liter:**

Agar.....	13.5g
L-Lysine.....	10.0g
Pancreatic digest of gelatin.....	5.0g
Yeast extract.....	3.0g
Glucose.....	1.0g
Ferric ammonium citrate.....	0.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	0.04g
Bromcresol Purple.....	0.02g

pH 6.7 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 12 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Use:** For the cultivation and differentiation of members of the Enterobacteriaceae based on their ability to decarboxylate lysine and to form H<sub>2</sub>S. Bacteria that decarboxylate lysine turn the medium purple. Bacteria that produce H<sub>2</sub>S appear as black colonies.

**M3 Agar**

**Composition per 1020.0mL:**

Agar.....	18.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.732g
KH <sub>2</sub> PO <sub>4</sub> .....	0.466g
NaCl.....	0.29g
Sodium propionate.....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CaCO <sub>3</sub> .....	0.02g
KNO <sub>3</sub> .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	0.02mg
Cycloheximide solution.....	10.0mL
Thiamine-HCl solution.....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Cycloheximide Solution:**

**Composition per 10.0mL:**

Cycloheximide.....	0.05g
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**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Thiamine-HCl Solution:**

**Composition per 10.0mL:**

Thiamine-HCl.....	4.0mg
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**Preparation of Thiamine-HCl Solution:** Add thiamine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cycloheximide solution and thiamine-HCl solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile cycloheximide solution and 10.0mL of thiamine-HCl solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of *Nocardia* species and *Rhodococcus* species.

**M Medium**

**Composition per liter:**

Beef.....	5.0g
Neopeptone.....	4.0g
NaCl.....	1.6g
Glucose.....	0.5g
CaCl <sub>2</sub> .....	0.06g
KH <sub>2</sub> PO <sub>4</sub> .....	0.06g
KCl.....	0.04g
Rabbit blood solution.....	200.0mL

**Rabbit Blood Solution:**

**Composition per liter:**

Rabbit blood.....	500.0mL
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**Preparation of Rabbit Blood Solution:** Add 500.0mL of whole rabbit blood to 500.0mL of sterile distilled/deionized water. Freeze and thaw twice to lyse blood cells.

**Preparation of Medium:** Trim beef to remove fat. Add 5.0g of lean beef to 200.0mL of distilled/deionized water. Gently heat and bring to boiling. Boil for 2–3 min. Filter through Whatman #2 filter paper. Add other components, except rabbit blood solution. Bring volume to 800.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 7.2 with 1N NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 200.0mL



of lysed rabbit blood solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Herpetomonas megaseliae*.

### M1 Medium

#### Composition per liter:

L-Leucine	2.0g
L-Alanine	1.0g
L-Isoleucine	1.0g
L-Phenylalanine	1.0g
L-Proline	1.0g
L-Tryptophane	1.0g
L-Asparagine	0.5g
L-Lysine	0.5g
L-Methionine	0.5g
L-Tyrosine	0.4g
L-Valine	0.2g
L-Serine	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
NaCl	0.2g
KH <sub>2</sub> PO <sub>4</sub>	0.14g
L-Arginine	0.1g
L-Cysteine	0.1g
L-Glycine	0.1g
L-Histidine	0.1g
L-Threonine	0.1g
CaCl <sub>2</sub>	2.0mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O	2.0mg
Tris(hydroxymethyl)aminomethane buffer (0.01M solution, pH 7.6)	1.0L
pH 7.6 ± 0.2 at 25°C	

**Preparation of Medium:** Add solid components to 1.0L of Tris buffer. Mix thoroughly. Filter sterilize. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation of *Myxococcus xanthus*.

### M14 Medium

#### Composition per liter:

Yeast extract	1.0g
D-Glucose	1.0g
Tris(hydroxymethyl)aminomethane	0.753g
Artificial seawater	250.0mL
Modified Hutner's basal salts	20.0mL
pH 7.5 ± 0.2 at 25°C	

#### Artificial Seawater:

##### Composition per liter:

NaCl	23.48g
MgCl <sub>2</sub>	4.98g
Na <sub>2</sub> SO <sub>4</sub>	3.92g
CaCl <sub>2</sub>	1.1g
KCl	0.66g
NaHCO <sub>3</sub>	0.19g
H <sub>3</sub> BO <sub>3</sub>	0.026g
SrCl <sub>2</sub>	0.024g
KBr	6.0mg
NaF	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Modified Hutner's Basal Salts:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	29.7g
Nitritotriacetic acid	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	99.0mg

(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub>	9.25mg
"Metals 44"	50.0mL

#### Preparation of Modified Hutner's Basal Salts:

Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### "Metals 44":

##### Composition per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
EDTA	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except modified Hutner's basal salts, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 20.0mL of sterile modified Hutner's basal salts. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pirellula marina*.

### M17 Medium

#### for *Filomicrobium fusiforme* (DSMZ Medium 768)

##### Composition per liter:

Na-acetate	1.0g
KNO <sub>3</sub>	1.0g
Artificial seawater, concentrated	500.0mL
Hutner's salts	20.0mL
Vitamin solution	10.0mL
pH 7.2 ± 0.2 at 25°C	

#### Hutner's Salts Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	29.7g
Nitritotriacetic acid	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	9.25mg
"Metals 44"	50.0mL

#### "Metals 44":

##### Composition per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
Sodium EDTA	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Salts Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust

pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### Artificial Seawater, Concentrated:

##### Composition per liter:

NaCl .....	70.43g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	31.86g
Na <sub>2</sub> SO <sub>4</sub> .....	11.75g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	4.35g
NaHCO <sub>3</sub> .....	2.88g
KCl .....	1.99g
KBr .....	0.29g
H <sub>3</sub> BO <sub>3</sub> .....	0.08g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Vitamin Solution:

##### Composition per liter:

Riboflavin .....	5.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Ca-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except artificial seawater and vitamin solution, to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 500.0mL artificial seawater and 10.0mL vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Filomicrobium fusiforme*.

#### M13 *Verrucomicrobium* Medium

##### Composition per liter:

Glucose .....	0.25g
Peptone .....	0.25g
Yeast extract .....	0.25g
Distilled water .....	670.0mL
Artificial seawater .....	250.0mL
Tris-HCl buffer, (0.1M solution, pH 7.5) .....	50.0mL
Modified Huntner's basal salts .....	20.0mL
Vitamin solution .....	10.0mL

pH 7.5 ± 0.2 at 25°C

#### Artificial Seawater:

##### Composition per liter:

NaCl .....	23.48g
MgCl <sub>2</sub> .....	4.98g
Na <sub>2</sub> SO <sub>4</sub> .....	3.92g
CaCl <sub>2</sub> .....	1.1g
KCl .....	0.66g
NaHCO <sub>3</sub> .....	0.19g
H <sub>3</sub> BO <sub>3</sub> .....	0.026g
SrCl <sub>2</sub> .....	0.024g
KBr .....	6.0mg
NaF .....	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Modified Hutner's Basal Salts:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

#### Preparation of Modified Hutner's Basal Salts:

Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### "Metals 44":

##### Composition per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

D-Calcium pantothenate .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except modified Hutner's basal salts, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 20.0mL of sterile modified Hutner's basal salts. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Verrucomicrobium spinosum*.

#### M1A Medium

##### Composition per 1001.0mL:

Sorbitol .....	23.3g
Peptone .....	6.0g
Sucrose .....	3.3g
Pancreatic digest of casein .....	3.3g
Beef heart infusion .....	2.0g
Glucose .....	1.3g
Yeast extract .....	1.0g
Fructose .....	0.3g
Phenol Red .....	20.0mg
Schneider's <i>Drosophila</i> medium .....	533.0mL
Fetal calf serum, heat inactivated .....	167.0mL
Fresh yeast extract solution .....	33.0mL
Penicillin solution .....	8.0mL

#### Schneider's *Drosophila* Medium:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.7g
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NaCl .....	2.1g
Yeast extract .....	2.0g
Trehalose .....	2.0g
D-Glucose .....	2.0g
L-Glutamine .....	1.8g
L-Lysine-HCl .....	1.7g
L-Proline .....	1.7g
KCl .....	1.6g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	1.3g
L-Glutamic acid .....	0.8g
L-Methionine .....	0.8g
CaCl <sub>2</sub> , anhydrous .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
β-Alanine .....	0.5g
L-Tyrosine .....	0.5g
L-Arginine .....	0.4g
L-Aspartic acid .....	0.4g
L-Histidine .....	0.4g
L-Threonine .....	0.4g
NaHCO <sub>3</sub> .....	0.4g
Glycine .....	0.3g
L-Serine .....	0.3g
L-Valine .....	0.3g
L-Isoleucine .....	0.2g
L-Leucine .....	0.2g
L-Phenylalanine .....	0.2g
α-Ketoglutaric acid .....	0.2g
Fumaric acid .....	0.1g
Malic acid .....	0.1g
Succinic acid .....	0.1g
L-Cystine .....	0.1g
L-Tryptophan .....	0.1g
L-Cysteine .....	0.06g

**Preparation of Schneider's *Drosophila* Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### **Penicillin Solution:**

**Composition per 10.0mL:**

Penicillin .....	2,500,000U
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**Preparation of Penicillin Solution:** Add penicillin to distilled/deionized water and bring volume to 10.0mL. Filter sterilize.

#### **Fresh Yeast Extract Solution:**

**Composition per 100.0mL:**

Baker's yeast, live, pressed, starch-free .....	25.0g
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**Preparation of Fresh Yeast Extract Solution:** Add the live Baker's yeast to 100.0mL of distilled/deionized water. Autoclave for 90 min at 15 psi pressure–121°C. Allow to stand. Remove supernatant solution. Adjust pH to 6.6–6.8. Filter sterilize.

**Preparation of Medium:** Add components—except Schneider's *Drosophila* medium, fetal calf serum, fresh yeast extract solution, and penicillin solution—to distilled/deionized water and bring volume to 260.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 533.0mL of sterile Schneider's *Drosophila* medium, 167.0mL of sterile fetal calf serum, 33.0mL of sterile fresh yeast extract solution, and 8.0mL of sterile penicillin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Spiroplasma* species that cause corn stunt.

### **mAB1 Medium**

**Composition per 1003.0mL:**

NaCl .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.25g
Yeast extract .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Sodium benzoate .....	0.15g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
Wolfe's vitamin solution .....	20.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> SeO <sub>3</sub> /Na <sub>2</sub> WO <sub>4</sub> solution .....	1.0mL
Sodium dithionite solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### **Wolfe's Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### **NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>SeO<sub>3</sub>/Na<sub>2</sub>WO<sub>4</sub> Solution:**

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Na<sub>2</sub>SeO<sub>3</sub>/Na<sub>2</sub>WO<sub>4</sub> Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Sodium Dithionite Solution:**

**Composition per 10.0mL:**

Sodium dithionite .....	0.2g
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**Preparation of Sodium Dithionite Solution:** Add sodium dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare medium and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Wolfe's vitamin solution, NaHCO<sub>3</sub> solution, sodium dithionite solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na<sub>2</sub>SeO<sub>3</sub>/Na<sub>2</sub>WO<sub>4</sub> solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of Wolfe's vitamin solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 1.0mL Na<sub>2</sub>SeO<sub>3</sub>/Na<sub>2</sub>WO<sub>4</sub> solution, 1.0mL of sterile sodium dithionite solution, and 1.0mL of sterile trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfotomaculum* species.

**MacConkey Agar****Composition per liter:**

Pancreatic digest of gelatin .....	17.0g
Agar .....	13.5g
Lactose .....	10.0g
NaCl .....	5.0g
Bile salts .....	1.5g
Pancreatic digest of casein .....	1.5g
Peptic digest of animal tissue .....	1.5g
Neutral Red .....	0.03g
Crystal Violet .....	1.0mg

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring until boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation, cultivation, and differentiation of coliforms and enteric pathogens based on the ability to ferment lactose. Lactose-fermenting organisms appear as red to pink colonies. Lactose-nonfermenting organisms appear as colorless or transparent colonies.

**MacConkey Agar****Composition per liter:**

Peptone .....	20.0g
Agar .....	12.0g
Lactose .....	10.0g

Bile salts .....	5.0g
NaCl .....	5.0g
Neutral Red .....	0.075g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring until boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation, cultivation, and differentiation of coliforms and enteric pathogens based on the ability to ferment lactose. Lactose-fermenting organisms appear as red-to-pink colonies. Lactose-nonfermenting organisms appear as colorless or transparent colonies.

**MacConkey Agar, CS****Composition per liter:**

Peptone .....	17.0g
Agar .....	13.5g
Lactose .....	10.0g
NaCl .....	5.0g
Proteose peptone .....	3.0g
Bile salts .....	1.5g
Neutral Red .....	0.03g
Crystal Violet .....	1.0mg

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available as a prepared medium from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring until boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and differentiation of lactose-fermenting and nonfermenting Gram-negative bacteria while also controlling the swarming of *Proteus* species, if present. Lactose-fermenting organisms appear as red-to-pink colonies. Lactose-nonfermenting organisms appear as colorless or transparent colonies.

**MacConkey Broth****Composition per liter:**

Pancreatic digest of gelatin .....	20.0g
Lactose .....	10.0g
Oxgall .....	5.0g
Bromocresol Purple .....	0.02g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. If testing 10.0mL samples, prepare medium double strength. Mix thoroughly. Gently heat while stirring until boiling. Distribute into test tubes containing inverted Durham tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the selective isolation and cultivation of coliforms in water.

**MacConkey Broth****Composition per liter:**

Peptone .....	20.0g
Lactose .....	10.0g

Bile salts.....	5.0g
NaCl.....	5.0g
Neutral Red.....	0.075g
pH 7.4 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. If testing 10.0mL samples, prepare medium double strength. Mix thoroughly. Gently heat while stirring until boiling. Distribute into test tubes containing inverted Durham tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the selective isolation and cultivation of coliforms in water.

### MacConkey Broth, Purple

**Composition per liter:**

Peptone.....	20.0g
Lactose.....	10.0g
Bile salts.....	5.0g
NaCl.....	5.0g
Bromcresol Purple.....	0.01g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder or tablets from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. If testing 10.0mL samples, prepare medium double strength. Mix thoroughly. Gently heat while stirring until boiling. Distribute into test tubes containing inverted Durham tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the selective isolation and cultivation of coliforms in water.

### Magnetic *Spirillum* Growth Medium, Revised (MSGM, Revised)

**Composition per liter:**

Agar.....	1.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.68g
Tartaric acid.....	0.37g
Succinic acid.....	0.37g
NaNO <sub>3</sub> .....	0.12g
Sodium acetate.....	0.05g
Ascorbic acid.....	0.035g
Wolfe's vitamin solution.....	10.0mL
Wolfe's mineral solution.....	5.0mL
Ferric quinate solution.....	2.0mL
Resazurin (0.1% solution).....	0.45mL

pH 6.75 ± 0.2 at 25°C

### Wolfe's Vitamin Solution:

**Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
Calcium pantothenate.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Wolfe's Mineral Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitriloacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

### Ferric Quinate Solution:

**Composition per 100.0mL:**

FeCl <sub>3</sub> .....	0.27g
Quinic acid.....	0.19g

**Preparation of Ferric Quinate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 1.0L of distilled/deionized water add components in the following order: Wolfe's vitamin solution, Wolfe's mineral solution, ferric quinate solution, resazurin, KH<sub>2</sub>PO<sub>4</sub>, NaNO<sub>3</sub>, ascorbic acid, tartaric acid, succinic acid, sodium acetate, and agar. Mix thoroughly after each addition. Adjust pH to 6.75 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically distribute into sterile screw-capped tubes. Fill tubes to capacity with medium. Use a heavy inoculum in each tube and do not introduce a headspace of air. Screw down caps tightly.

**Use:** For the cultivation and maintenance of *Aquaspirillum magnetotacticum*.

### Magnetospirillum Medium (DSMZ Medium 380)

**Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	0.68g
L(+)-Tartaric acid.....	0.37g
Succinic acid.....	0.37g
NaNO <sub>3</sub> .....	0.12g
Na-thioglycolate.....	0.05g
Na-acetate.....	0.05g
Resazurin.....	0.5mg
Vitamin solution.....	10.0mL
Trace elements solution.....	5.0mL
Ferric quinate solution.....	2.0mL

pH 6.8 ± 0.2 at 25°C

### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Ferric Quinate Solution:

##### Composition per 100.0mL:

FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.45g
Quinic acid.....	0.19g

**Preparation of Ferric Quinate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except vitamin solution and ferric quinate solution, to distilled/deionized water and bring volume to 988.0mL. Purge medium with N<sub>2</sub> gas for 10 min. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL vitamin solution and 2.0mL ferric quinate solution. Mix thoroughly. Purge medium with N<sub>2</sub> gas for 10 min. Under the same atmosphere, anaerobically fill tubes to 1/3 of their volume and seal. Autoclave at 121°C for 15 min. Before inoculation, add sterile air (with hypodermic syringe through the rubber closure) to 1% O<sub>2</sub> concentration in the gas phase.

**Use:** For the cultivation of *Magnetospirillum magnetotacticum*=*Aquaspirillum magnetotacticum*, and *Magnetospirillum gryphiswaldense*.

#### Magnetospirillum 2 Medium

##### Composition per liter:

Sodium acetate.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Sodium thioglycolate.....	0.5g
NH <sub>4</sub> Cl.....	0.1g
Yeast extract.....	0.1g
Ferric citrate.....	20.0μg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into screw-capped tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow medium to stand upright at room temperature for 2 to 3 days before inoculation. Do not shake.

**Use:** For the cultivation and maintenance of *Magnetospirillum gryphiswaldense*.

#### Magnetospirillum Semi-Solid Medium (DSMZ Medium 380)

##### Composition per liter:

Agar.....	1.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.68g
L(+)-Tartaric acid.....	0.37g
Succinic acid.....	0.37g
NaNO <sub>3</sub> .....	0.12g
Na-thioglycolate.....	0.05g
Na-acetate.....	0.05g
Resazurin.....	0.5mg
Vitamin solution.....	10.0mL
Trace elements solution.....	5.0mL
Ferric quinate solution.....	2.0mL

pH 6.8 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Ferric Quinate Solution:

##### Composition per 100.0mL:

FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.45g
Quinic acid.....	0.19g

**Preparation of Ferric Quinate Solution:** Add components to distilled/deionized water and bring volume to

100.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C.

**Preparation of Medium:** Add components, except vitamin solution and ferric quinate solution, to distilled/deionized water and bring volume to 988.0mL. Purge medium with N<sub>2</sub> gas for 10 min. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically and anaerobically add 10.0mL vitamin solution and 2.0mL ferric quinate solution. Mix thoroughly. Purge medium with N<sub>2</sub> gas for 10 min. Dispense 12mL of medium per 16 x 150mm anaerobe screw cap tube under N<sub>2</sub> gas. Prior to inoculation, remove caps briefly under air, tighten the caps again and wait several hours to establish oxygen gradients. The medium should be slightly pink in color. Strongly reduced conditions will not support growth of the organism. During growth O<sub>2</sub> will be consumed, resazurin decolorized, and the pH increased. Feed oxygen (by adding air) and succinic acid from sterile 0.05M solution (to maintain pH below 7.0). If higher densities of magnetic cell are wanted, ferric quinate also can be fed. For transfer use cell material which has been concentrated at the glass wall of the culture vessel by means of a magnetic rod attached outside.

**Use:** For the cultivation of *Magnetospirillum magnetotacticum*=*Aquaspirillum magnetotacticum*, and *Magnetospirillum gryphiswaldense*.

#### Malachite Green Broth

##### Composition per liter:

Peptone.....	15.0g
Beef extract.....	9.0g
Malachite Green.....	0.01mg

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas aeruginosa*.

#### Malonate Broth

##### Composition per liter:

Sodium malonate.....	3.0g
NaCl.....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
Bromothymol Blue.....	0.025g

pH 6.7 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Avoid introduction of carbon and nitrogen from other sources.

**Use:** For the cultivation and differentiation of coliforms and other enteric organisms, particularly *Enterobacter* and *Escherichia*, based on their ability to utilize malonate as the sole carbon source and ammonium sulfate as the sole nitrogen source. Malonate-utilizing organisms turn the medium blue.

#### Malonate Broth, Ewing Modified

##### Composition per liter:

Sodium malonate.....	3.0g
NaCl.....	2.0g

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Yeast extract.....	1.0g
Glucose.....	0.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
Bromothymol Blue.....	0.025g

pH 6.7 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of coliforms and other enteric organisms, particularly *Enterobacter* and *Escherichia*, based on their ability to utilize malonate as a carbon source and ammonium sulfate as a nitrogen source. The small amount of yeast extract and glucose encourages the growth of some organisms that may be distressed or fail to respond. Malonate-utilizing organisms turn the medium blue.

#### Malt Agar

##### Composition per liter:

Malt extract.....	30.0g
Agar.....	15.0g

pH 5.5 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring until boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–118°C. Do not overheat or agar will not harden. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of yeasts and molds.

#### Manganese Acetate Agar

##### Composition per liter:

Agar, highly purified.....	10.0g
Manganous acetate.....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add manganous acetate to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Add agar. Steam the medium to dissolve agar. Distribute into screw-capped tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes or bottles to cool in a slanted position.

**Use:** For the cultivation of manganese-oxidizing bacteria.

#### Manganese Agar No. 1 (Mn Agar No. 1)

##### Composition per liter:

Agar.....	10.0g
MnCO <sub>3</sub> .....	2.0g
Beef extract.....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	0.15g
Sodium citrate.....	0.15g
Yeast extract.....	0.075g
Cyanocobalamin solution.....	10.0mL

##### Cyanocobalamin Solution:

##### Composition per 10.0mL:

Cyanocobalamin.....	0.005mg
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**Preparation of Cyanocobalamin Solution:** Add cyanocobalamin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cyanocobalamin, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of the sterile cyanocobalamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of iron and sulfur bacteria. Also used to differentiate *Leptothrix* (*Sphaerotilus*) *discophorus* from *Sphaerotilus natans*.

### Manganese Agar No. 2 (Mn Agar No. 2)

**Composition per liter:**

Agar .....	15.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	10.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Use freshly prepared solution.

**Use:** For the enumeration, enrichment, and isolation of iron and sulfur bacteria. For the isolation and cultivation of *Leptothrix* species from water.

### Manganese Medium for *Pseudomonas* species

**Composition per liter:**

Noble agar .....	10.0g
MnCO <sub>3</sub> .....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.15g
Sodium citrate .....	0.15g
Yeast extract .....	0.075g
Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.05g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas putida* and other *Pseudomonas* species.

### Mannitol Yeast Extract Medium (LMG 135)

**Composition per liter:**

Agar .....	20.0g
Mannitol .....	10.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution .....	1.0mL
FeCl <sub>3</sub> ·6H <sub>2</sub> O solution .....	1.0mL

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.28mg
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**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add 5.28g of CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**FeCl<sub>3</sub>·6H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.66mg
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**Preparation of FeCl<sub>3</sub>·6H<sub>2</sub>O Solution:** Add FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Rhizobium* species and *Bradyrhizobium* species.

### Marine Agar (DSMZ Medium 123)

**Composition per liter:**

Agar .....	15.0g
Tryptone .....	10.0g
Peptone .....	5.0g
Yeast extract .....	1.0g
Synthetic seawater .....	1.0L

pH 7.8 ± 0.2 at 25°C

**Synthetic Seawater:**

**Composition per liter:**

NaCl .....	24.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	11.0g
Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0g
KCl .....	0.7g
KBr .....	0.1g
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.04g
H <sub>3</sub> BO <sub>3</sub> .....	0.03g
NaSiO <sub>3</sub> ·9H <sub>2</sub> O .....	5.0mg
NaF .....	3.0mg
NH <sub>4</sub> NO <sub>3</sub> .....	2.0mg
Fe <sub>3</sub> PO <sub>4</sub> ·4H <sub>2</sub> O .....	1.0mg

**Preparation of Synthetic Seawater:** Add components to distilled water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add agar, tryptone, peptone, and yeast extract to synthetic seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.8. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Halobacillus halophilus*, *Halomonas* spp., *Vibrio harveyi*, *Cobetia marina*, and *Ruegeria atlantica*.

### Marine Agar 2216 (DSMZ Medium 604)

**Composition per liter:**

NaCl .....	19.45g
Agar .....	15.0g
MgCl <sub>2</sub> .....	8.8g
Peptone .....	5.0g
Na <sub>2</sub> SO <sub>3</sub> .....	3.24g
CaCl <sub>2</sub> .....	1.8g
Yeast extract .....	1.0g
KCl .....	0.55g
NaHCO <sub>3</sub> .....	0.16g
Ferric citrate .....	0.1g
KBr .....	0.08g
SrCl <sub>2</sub> .....	0.03g
H <sub>3</sub> BO <sub>3</sub> .....	0.02g



Na <sub>2</sub> HPO <sub>4</sub> .....	8.0mg
Na <sub>2</sub> SiO <sub>3</sub> .....	4.0mg
NaF.....	2.4mg
NH <sub>4</sub> NO <sub>3</sub> .....	1.6mg

pH 7.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Hyphomonas* spp., *Oceanospirillum* spp., *Hyphomicrobium indicum*, *Psychroflexus gondwanensis*=*Flavobacterium gondwanense*, *Salegentibacter salegens*=*Flavobacterium salegens*, *Psychromonas antarctica*, *Sulfotobacter mediterraneus*, *Thalassomonas viridans*, *Vibrio* spp., *Marinospirillum minutulum*=*Oceanospirillum minutulum*, *Terasakiella pusilla*=*Oceanospirillum pusillum*, *Pseudoalteromonas atlantica*=*Alteromonas atlantica*, *Pseudomonas atlantica*, *Roseobacter* spp., *Erythrobacter longus*, *Pseudospirillum japonicum*=*Oceanospirillum japonicum*, *Marinobacter hydrocarbonoclasticus* (*Pseudomonas nautica*), *Psychrobacter* spp., and *Moritella japonica*. For the isolation, cultivation, and maintenance of a wide variety of heterotrophic marine bacteria.

### Marine Agar with Biphenyl

#### Composition per liter:

NaCl.....	19.45g
Agar.....	15.0g
MgCl <sub>2</sub> .....	8.8g
Peptone.....	5.0g
Na <sub>2</sub> SO <sub>3</sub> .....	3.24g
CaCl <sub>2</sub> .....	1.8g
Yeast extract.....	1.0g
KCl.....	0.55g
NaHCO <sub>3</sub> .....	0.16g
Ferric citrate.....	0.1g
KBr.....	0.08g
SrCl <sub>2</sub> .....	0.03g
H <sub>3</sub> BO <sub>3</sub> .....	0.02g
Na <sub>2</sub> HPO <sub>4</sub> .....	8.0mg
Na <sub>2</sub> SiO <sub>3</sub> .....	4.0mg
NaF.....	2.4mg
NH <sub>4</sub> NO <sub>3</sub> .....	1.6mg
Biphenyl.....	1.0mg

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except biphenyl, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. After agar solidifies, aseptically add a few crystals of biphenyl to each plate.

**Use:** For the cultivation and maintenance of biphenyl-utilizing marine bacteria, such as *Cyclocloasticus pugetii*,

### Marine Agar with κ- and λ-Carrageenan

#### Composition per 1070.0mL:

Solution A.....	1.0L
Solution B.....	60.0mL
Solution C.....	10.0mL

pH 7.2 ± 0.2 at 25°C

### Solution A:

#### Composition per liter:

NaCl.....	25.0g
Agar.....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0g
Casamino acids.....	2.5g
NaNO <sub>3</sub> .....	2.0g
κ-Carrageenan.....	1.25g
λ-Carrageenan.....	1.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.2g
KCl.....	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution B:

#### Composition per 100.0mL:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	3.56g
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**Preparation of Solution B:** Add component to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

### Solution C:

#### Composition per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.3g
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**Preparation of Solution C:** Add component to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically add 60.0mL of sterile solution B and 10.0mL of sterile solution C to 1.0L of sterile solution A. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas carrageenovora*.

### Marine Agar with Naphthalene

#### Composition per liter:

NaCl.....	19.45g
Agar.....	15.0g
MgCl <sub>2</sub> .....	8.8g
Peptone.....	5.0g
Na <sub>2</sub> SO <sub>3</sub> .....	3.24g
CaCl <sub>2</sub> .....	1.8g
Yeast extract.....	1.0g
KCl.....	0.55g
NaHCO <sub>3</sub> .....	0.16g
Ferric citrate.....	0.1g
KBr.....	0.08g
SrCl <sub>2</sub> .....	0.03g
H <sub>3</sub> BO <sub>3</sub> .....	0.02g
Na <sub>2</sub> HPO <sub>4</sub> .....	8.0mg
Na <sub>2</sub> SiO <sub>3</sub> .....	4.0mg
NaF.....	2.4mg
NH <sub>4</sub> NO <sub>3</sub> .....	1.6mg
Naphthalene.....	1mg

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except naphthalene, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. After agar solidifies, aseptically add a few crystals of naphthalene to each plate.

**Use:** For the cultivation and maintenance of naphthalene-utilizing marine bacteria.

### Marine Agar with Sulfur (ATCC Medium 1922)

#### Composition per liter:

NaCl	19.45g
Sulfur	10.0g
MgCl <sub>2</sub>	8.8g
Peptone	5.0g
Na <sub>2</sub> SO <sub>3</sub>	3.24g
CaCl <sub>2</sub>	1.8g
Yeast extract	1.0g
KCl	0.55g
NaHCO <sub>3</sub>	0.16g
Ferric citrate	0.1g
KBr	0.08g
SrCl <sub>2</sub>	0.03g
H <sub>3</sub> BO <sub>3</sub>	0.02g
Na <sub>2</sub> HPO <sub>4</sub>	8.0mg
Na <sub>2</sub> SiO <sub>3</sub>	4.0mg
NaF	2.4mg
NH <sub>4</sub> NO <sub>3</sub>	1.6mg

pH 7.6  $\pm$  0.2 at 25°C

**Preparation of Sulfur:** Autoclave sulfur for 15 min at 0 psi pressure–100°C on 3 successive days.

**Preparation of Medium:** Prepare anaerobically under a gas phase of 80% N<sub>2</sub> + 10% CO<sub>2</sub> + 10% H<sub>2</sub>. Add components, except sulfur, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50–55°C. Aseptically add 10.0g of sterile sulfur. Mix thoroughly. Aseptically and anaerobically, under a gas phase of 80% N<sub>2</sub> + 10% CO<sub>2</sub> + 10% H<sub>2</sub>, distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermococcus litoralis*.

### Marine Broth 2216 (LMG Medium 164)

#### Composition per liter:

NaCl	19.45g
MgCl <sub>2</sub>	8.8g
Peptone	5.0g
Na <sub>2</sub> SO <sub>3</sub>	3.24g
CaCl <sub>2</sub>	1.8g
Yeast extract	1.0g
KCl	0.55g
NaHCO <sub>3</sub>	0.16g
Ferric citrate	0.1g
KBr	0.08g
SrCl <sub>2</sub>	0.03g
H <sub>3</sub> BO <sub>3</sub>	0.02g
Na <sub>2</sub> HPO <sub>4</sub>	8.0mg
Na <sub>2</sub> SiO <sub>3</sub>	4.0mg
NaF	2.4mg
NH <sub>4</sub> NO <sub>3</sub>	1.6mg

pH 7.6  $\pm$  0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Vibrio liquefaciens* and for the isolation, cultivation, and maintenance of a wide variety of heterotrophic marine bacteria.

### Marine Broth with Biphenyl

#### Composition per liter:

NaCl	19.45g
MgCl <sub>2</sub>	8.8g
Peptone	5.0g
Na <sub>2</sub> SO <sub>3</sub>	3.24g
CaCl <sub>2</sub>	1.8g
Yeast extract	1.0g
KCl	0.55g
NaHCO <sub>3</sub>	0.16g
Ferric citrate	0.1g
KBr	0.08g
SrCl <sub>2</sub>	0.03g
H <sub>3</sub> BO <sub>3</sub>	0.02g
Na <sub>2</sub> HPO <sub>4</sub>	8.0mg
Na <sub>2</sub> SiO <sub>3</sub>	4.0mg
NaF	2.4mg
NH <sub>4</sub> NO <sub>3</sub>	1.6mg
Biphenyl	1.0mg

pH 7.6  $\pm$  0.2 at 25°C

**Preparation of Medium:** Add components, except biphenyl, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add a few crystals of biphenyl to each tube or flask.

**Use:** For the cultivation of biphenyl-utilizing marine bacteria.

### Marine Broth with $\kappa$ - and $\lambda$ -Carrageenan

#### Composition per 1070.0mL:

Solution A	1.0L
Solution B	60.0mL
Solution C	10.0mL

pH 7.2  $\pm$  0.2 at 25°C

#### Solution A:

##### Composition per liter:

NaCl	25.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	5.0g
Casamino acids	2.5g
NaNO <sub>3</sub>	2.0g
$\kappa$ -Carrageenan	1.25g
$\lambda$ -Carrageenan	1.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.2g
KCl	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B:

##### Composition per 100.0mL:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O	3.56g
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**Preparation of Solution B:** Add component to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

##### Composition per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.3g
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**Preparation of Solution C:** Add component to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically add 60.0mL of sterile solution B and 10.0mL of sterile solution C to 1.0L

of sterile solution A. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudomonas carraegenovora*.

### Marine Broth with Naphthalene

**Composition per liter:**

NaCl	19.45g
MgCl <sub>2</sub>	8.8g
Peptone	5.0g
Na <sub>2</sub> SO <sub>3</sub>	3.24g
CaCl <sub>2</sub>	1.8g
Yeast extract	1.0g
KCl	0.55g
NaHCO <sub>3</sub>	0.16g
Ferric citrate	0.1g
KBr	0.08g
SrCl <sub>2</sub>	0.03g
H <sub>3</sub> BO <sub>3</sub>	0.02g
Na <sub>2</sub> HPO <sub>4</sub>	8.0mg
Na <sub>2</sub> SiO <sub>3</sub>	4.0mg
NaF	2.4mg
NH <sub>4</sub> NO <sub>3</sub>	1.6mg
Naphthalene	1mg

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except biphenyl, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add a few crystals of naphthalene to each tube or flask.

**Use:** For the cultivation of naphthalene-utilizing marine bacteria.

### Marine Broth with Sulfur

**Composition per liter:**

NaCl	19.45g
Sulfur	10.0g
MgCl <sub>2</sub>	8.8g
Peptone	5.0g
Na <sub>2</sub> SO <sub>3</sub>	3.24g
CaCl <sub>2</sub>	1.8g
Yeast extract	1.0g
KCl	0.55g
NaHCO <sub>3</sub>	0.16g
Ferric citrate	0.1g
KBr	0.08g
SrCl <sub>2</sub>	0.03g
H <sub>3</sub> BO <sub>3</sub>	0.02g
Na <sub>2</sub> HPO <sub>4</sub>	8.0mg
Na <sub>2</sub> SiO <sub>3</sub>	4.0mg
NaF	2.4mg
NH <sub>4</sub> NO <sub>3</sub>	1.6mg

pH 7.6 ± 0.2 at 25°C

**Preparation of Sulfur:** Autoclave for 15 min at 0 psi pressure–100°C on 3 successive days.

**Preparation of Medium:** Prepare anaerobically under a gas phase of 80% N<sub>2</sub> + 10% CO<sub>2</sub> + 10% H<sub>2</sub>. Add components, except sulfur, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0g of sulfur. Mix thoroughly. Aseptically and anaerobically, under a gas phase of 80% N<sub>2</sub> + 10% CO<sub>2</sub> + 10% H<sub>2</sub>, distribute into sterile tubes.

**Use:** For the cultivation of *Thermococcus litoralis*.

### Marine *Caulobacter* Medium

**Composition per liter:**

Proteose peptone	10.0g
Yeast extract	3.0g
Artificial seawater	1.0L

pH 7.2–7.4 at 25°C

**Artificial Seawater:**

**Composition per liter:**

Commercially available marine aquarium salts mixture	variable
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**Preparation of Artificial Seawater:** Add commercially available marine aquarium salts mixture to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Caulobacter halobacteroides* and *Caulobacter maris*.

### Marine Chlorobiaceae Medium 2

**Composition per 1051.0mL:**

Solution 1	950.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	60.0mL
NaHCO <sub>3</sub> solution	40.0mL
Vitamin B <sub>12</sub> solution	1.0mL

pH 6.8 ± 0.2 at 25°C

**Solution 1:**

**Composition per 950.0mL:**

NaCl	20.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
KH <sub>2</sub> PO <sub>4</sub>	1.0g
NH <sub>4</sub> Cl	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Trace elements solution SL-8	1.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution SL-8:**

**Composition per liter:**

Disodium EDTA	5.2g
FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.1g
ZnCl <sub>2</sub>	0.07g
H <sub>3</sub> BO <sub>3</sub>	0.06g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	5.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**NaHCO<sub>3</sub> Solution:**

**Composition per 100.0mL:**

NaHCO <sub>3</sub>	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B<sub>12</sub> ..... 2.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 950.0mL of cooled, sterile solution 1, aseptically add 60.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 40.0mL of sterile NaHCO<sub>3</sub> solution, and 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Adjust pH to 6.8 with sterile H<sub>2</sub>SO<sub>4</sub> or Na<sub>2</sub>CO<sub>3</sub>. Aseptically distribute into sterile 50.0mL or 100.0mL bottles with metal screw-caps and rubber seals. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the isolation and cultivation of marine members of the Chlorobiaceae.

**Marine Chromatiaceae Medium 2**

**Composition** per 1051.0mL:

Solution 1 ..... 950.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 60.0mL  
NaHCO<sub>3</sub> solution ..... 40.0mL  
Vitamin B<sub>12</sub> solution ..... 1.0mL  
pH 7.3 ± 0.2 at 25°C

**Solution 1:**

**Composition** per 950.0mL:

NaCl ..... 20.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
NH<sub>4</sub>Cl ..... 0.5g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g  
Trace elements solution SL-8 ..... 1.0mL

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution SL-8:**

**Composition** per liter:

Disodium EDTA ..... 5.2g  
FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g  
ZnCl<sub>2</sub> ..... 0.07g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.06g  
NaMoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.04g  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.02g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02g

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 5.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B<sub>12</sub> ..... 2.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 950.0mL of cooled, sterile solution 1, aseptically add 60.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 40.0mL of sterile NaHCO<sub>3</sub> solution, and 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Adjust pH to 7.3 with sterile H<sub>2</sub>SO<sub>4</sub> or Na<sub>2</sub>CO<sub>3</sub>. Aseptically distribute into sterile 50.0mL or 100.0mL bottles with metal screw-caps and rubber seals. Completely fill bottles with medium except for a pea-sized air bubble.

**Use:** For the isolation and cultivation of marine members of the Chromatiaceae.

**Marine *Cytophaga* Agar**

**Composition** per liter:

Agar ..... 15.0g  
Nutrient broth ..... 8.0g  
Yeast extract ..... 5.0g  
Salt solution ..... 1.0L

**Salt Solution:**

**Composition** per liter:

NaCl ..... 12.86g  
MgCl<sub>2</sub> ..... 2.48g  
KCl ..... 0.75g  
CaCl<sub>2</sub> ..... 0.56g  
Fe(SO<sub>4</sub>)<sub>2</sub>(NH<sub>4</sub>)<sub>2</sub> ..... 0.048g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to 1.0L of salt solution. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Cytophaga* species.

**Marine *Cytophaga* Medium A**

**Composition** per liter:

Agar ..... 15.0g  
Pancreatic digest of casein ..... 2.0g  
Beef extract ..... 0.5g  
Yeast extract ..... 0.5g  
Sodium acetate ..... 0.2g  
Seawater ..... 700.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Flexibacter maritimus*.

**Marine *Cytophaga* Medium B**

**Composition** per liter:

Agar ..... 15.0g  
Pancreatic digest of casein ..... 2.0g

Beef extract .....	0.5g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g
Seawater .....	500.0mL
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Vibrio ordalii*.

#### **Marine Cytophaga Medium C**

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	2.0g
Beef extract .....	0.5g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to seawater and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Cytophaga agarovorans*, *Cytophaga fermentans*, and *Cytophaga salmonicolor*.

#### **Marine Desulfovibrio Medium**

**Composition** per liter:

Solution A .....	980.0mL
Solution B .....	10.0mL
Solution C .....	10.0mL
pH 7.8 ± 0.2 at 25°C	

##### **Solution A:**

**Composition** per 980.0mL:

NaCl .....	25.0g
DL-Sodium lactate .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 100% N<sub>2</sub>.

##### **Solution B:**

**Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
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**Preparation of Solution B:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

##### **Solution C:**

**Composition** per 10.0mL:

Ascorbic acid .....	0.1g
Sodium thioglycolate .....	0.1g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** To 980.0mL of cooled solution A, anaerobically add 10.0mL of solution B and 10.0mL of solution C. Mix thoroughly. Adjust pH to 7.8 with NaOH. Distribute into tubes or flasks. During distribution, swirl the medium to keep the precipitate in suspension. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Desulfovibrio desulfuricans*, *Desulfovibrio salexigens*, and *Desulfovibrio vulgaris*.

#### **Marine Flagellate Medium**

**Composition** per 15.0mL:

Rice grains .....	2.0g
Seawater .....	15.0mL

**Preparation of Medium:** Autoclave rice grains for 15 min at 15 psi pressure–121°C. Add 2.0g of sterile rice grains to 15.0mL of filter-sterilized seawater. Aseptically distribute into T-25 tissue culture flasks.

**Use:** For the cultivation of *Acanthoeopsis unguiculata*, *Amastigomonas* species, *Bicosoeca vacillans*, *Bodo designis*, *Bodo variabilis*, *Caecitellus parvulus*, *Choanoeca perplexa*, *Codosiga gracilis*, *Diaphanoeca grandis*, *Entosiphon* species, *Goniomonas* species, *Procryptobia* species, *Pseudobodo tremulans*, *Rhynchomonas nasuta*, *Salpingoeca urceolata*, *Stephanoeca diplocostata*, and *Stephanopogon apogon*.

#### **Marine Flagellate Medium with B-Vitamins**

**Composition** per liter:

Seawater .....	990.0mL
Vitamin solution .....	10.0mL

##### **Vitamin Solution:**

**Composition** per 100.0mL:

Thiamine-HCl .....	0.15g
Calcium D-(+)-pantothenate .....	0.05g
Nicotinamide .....	0.05g
Pyridoxal-HCl .....	0.05g
Riboflavin .....	0.05g
Folic acid .....	0.025g
Pyridoxamine-HCl .....	0.025g
Biotin .....	12.5mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Allow natural seawater to age for 2 months. Filter sterilize. Aseptically add 100.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Oikomonas* species.

#### **Marine Glucose Trypticase Yeast Extract Agar (MGTY Agar)**

**Composition** per liter:

Agar .....	8.0g
Glucose .....	2.0g
Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.5g
Seawater .....	750.0mL
Tris-HCl buffer (5.0 mM, pH 7.5) .....	50.0mL
Resazurin (0.1% solution) .....	1.0mL
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks under 97% N<sub>2</sub> + 3% H<sub>2</sub>. Cap with rubber stoppers and place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation and maintenance of *Spirochaeta isovalerica*.

### Marine Methanogenium Alcohol Medium

**Composition** per 1003.0mL:

NaCl .....	21.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.4g
Sodium acetate·3H <sub>2</sub> O .....	0.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
NaHCO <sub>3</sub> solution .....	60.0mL
2-Propanol .....	5.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	3.0mL
Cyanocobalamin solution .....	1.0mL
Selenite-molybdate-tungstate solution .....	1.0mL
Thiamine solution .....	1.0mL
Trace elements solution .....	1.0mL
Vitamin solution .....	1.0mL

### Trace Elements Solution:

**Composition** per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1400.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	145.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	120.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	50.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	3.0mg
HCl (25%,w/v) .....	8.0mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Selenite-Molybdate-Tungstate Solution:

**Composition** per liter:

NaOH .....	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	33.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Selenite-Molybdate-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### NaHCO<sub>3</sub> Solution:

**Composition** per liter:

NaHCO <sub>3</sub> .....	84.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.5g
NaOH .....	1 pellet

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Dissolve 1 pellet of NaOH in the anaerobic water. Weigh out a little more than 2.5g of Na<sub>2</sub>S·9H<sub>2</sub>O. Briefly rinse the crystals in distilled/deionized water. Dry the crystals by blotting on paper towels or filter paper. Add 2.5g of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals to 100.0mL of anaerobic NaOH solution. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Pressurize to 60kPa with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

**Preparation of 2-Propanol:** Filter sterilize 10.0mL of 2-propanol. Sparge with 100% N<sub>2</sub>.

### Vitamin Solution:

**Composition** per liter:

Sodium 2-mercaptoethanesulfonate .....	0.25g
Pyridoxine·HCl .....	0.15g
Calcium pantothenate .....	0.1g
Nicotinic acid .....	0.1g
<i>p</i> -Aminobenzoic acid .....	40.0mg
Biotin .....	10.0mg
Potassium phosphate buffer (25mM solution, pH 7.0) .....	1.0L

**Preparation of Vitamin Solution:** Combine components. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

### Thiamine Solution:

**Composition** per liter:

Thiamine·HCl .....	0.1g
Sodium phosphate buffer (0.1M solution, pH 3.6) .....	1.0L

**Preparation of Thiamine Solution:** Combine components. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

### Cyanocobalamin Solution:

**Composition** per liter:

Cyanocobalamin .....	50.0mg
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**Preparation of Cyanocobalamin Solution:** Add cyanocobalamin to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, 2-propanol, Na<sub>2</sub>S·9H<sub>2</sub>O solution, cyanocobalamin solution, selenite-molybdate-tungstate solution, thiamine solution, trace elements solution, and vitamin solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 60.0mL of sterile NaHCO<sub>3</sub> solution, 5.0mL of sterile 2-propanol, 3.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 1.0mL of sterile cyanocobalamin solution, 1.0mL of sterile selenite-molybdate-tungstate solution, 1.0mL of sterile thiamine solution, 1.0mL of sterile trace elements solution, and 1.0mL of sterile vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of marine *Methanogenium* species.

### Marine Methanol Medium

**Composition** per liter:

NaCl .....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g

K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.3g
Methanol.....	10.0mL
Vitamin B <sub>12</sub> solution.....	10.0mL
Trace metals solution.....	1.0mL
pH 7.0 ± 0.2 at 25°C	

**Vitamin B<sub>12</sub> Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	10.0μg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add the Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Adjust pH to 5. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Metals Solution:****Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.4g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.84g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.28g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.25g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.24g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.24g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g

**Preparation of Trace Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except Vitamin B<sub>12</sub> solution and methanol, to distilled/deionized water and bring volume to 980.0mL. Adjust pH to 7.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Filter sterilize methanol. Aseptically add sterile Vitamin B<sub>12</sub> solution and filter-sterilized methanol. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Methylophaga thalassica*.

**Marine Methylophaga Agar****Composition per 1003.0mL:**

Agarose.....	12.0g
Bis (2-hydroxyethyl) aminotris (hydroxy-methyl) methane.....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14g
Ferric ammonium citrate.....	0.06g
Methanol.....	2.0mL
Vitamin B <sub>12</sub> solution.....	1.0mL
pH 7.4 ± 0.2 at 25°C	

**Vitamin B<sub>12</sub> Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	0.1mg
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**Preparation of Vitamin Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Store at 5°C.

**Preparation of Medium:** Add components, except methanol and Vitamin B<sub>12</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 2.0mL of filter-sterilized methanol and 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Alteromonas* species, *Methylophaga marina*, *Methylophaga thalassica*, and *Methylophilus* species.

**Marine Peptone Succinate Salts Medium (PSS Medium)****Composition per liter:**

Peptone.....	10.0g
Succinic acid.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	2.0mg
Synthetic seawater.....	1.0L
pH 6.8 ± 0.2 at 25°C	

**Synthetic Seawater:****Composition per liter:**

NaCl.....	27.5g
MgCl <sub>2</sub> .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
KCl.....	1.0g
CaCl <sub>2</sub> .....	0.5g
FeSO <sub>4</sub> .....	1.0mg

**Preparation of Synthetic Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to 1.0L of synthetic seawater. Mix thoroughly. Gently heat while stirring and bring to boiling. Adjust pH to 6.8 with KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Oceanospirillum beijerinckii* and *Oceanospirillum multiglobuliferum*.

**Marine Peptone Yeast Medium with Magnesium Sulfate****Composition per liter:**

NaCl.....	20.0g
Peptone.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Yeast extract.....	1.0g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Oceanospirillum pusillum*.

**Marine Pseudomonas Medium****Composition per liter:**

Agar.....	15.0g
Nutrient broth.....	8.0g
Yeast extract.....	5.0g
Salt solution.....	1.0L

**Salt Solution:****Composition per liter:**

NaCl.....	12.86g
MgCl <sub>2</sub> .....	2.48g
KCl.....	0.75g
CaCl <sub>2</sub> .....	0.56g
Fe(SO <sub>4</sub> ) <sub>2</sub> (NH <sub>4</sub> ) <sub>2</sub> .....	0.048g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to 1.0L of salt solution. Mix thoroughly. Gently heat and bring to boil-

ing. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alteromonas haloplanktis*.

### Marine *Rhodococcus* Medium

**Composition** per liter:

Yeast extract	10.0g
Malt extract	4.0g
Glucose	4.0g
Seawater	750.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodococcus marionascens*.

### Marine *Rhodopseudomonas* Medium

**Composition** per liter:

NaCl	30.4g
Yeast extract	1.0g
Disodium succinate	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4g
NH <sub>4</sub> Cl	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Ferric citrate (0.1% solution)	5.0mL
Trace elements SL-6	1.0mL
Ethanol	0.5mL

pH 6.8 ± 0.2 at 25°C

### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodopseudomonas marina*.

### Marine Salts Medium

**Composition** per liter:

NaCl	81.0g
Yeast extract	10.0g
MgSO <sub>4</sub>	9.6g
MgCl <sub>2</sub>	7.0g
Proteose peptone No.3	5.0g
KCl	2.0g
Glucose	1.0g
CaCl <sub>2</sub>	0.36g

NaHCO <sub>3</sub>	0.06g
NaBr	0.026g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of marine bacteria.

### Marine Spirochete Medium

**Composition** per liter:

Cellobiose	2.0g
Peptone	2.0g
Yeast extract	1.0g
Sodium thioglycolate	1.0g
Seawater, charcoal filtered	800.0mL

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except sodium thioglycolate, to glass-distilled water and bring volume to 1.0L. Mix thoroughly. Bubble 100% N<sub>2</sub> into medium for 1.5 min. Add sodium thioglycolate. Adjust pH to 7.5 with 10N KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Spirochaeta bajacaliforniensis*.

### Marine *Thermococcus* Medium (DSMZ Medium 760)

**Composition** per liter:

NaCl	19.45g
MgCl <sub>2</sub>	8.8g
Sulfur	5.0g
Peptone	5.0g
Na <sub>2</sub> SO <sub>3</sub>	3.24g
CaCl <sub>2</sub>	1.8g
Yeast extract	1.0g
KCl	0.55g
NaHCO <sub>3</sub>	0.16g
Ferric citrate	0.1g
KBr	0.08g
SrCl <sub>2</sub>	0.03g
H <sub>3</sub> BO <sub>3</sub>	0.02g
Na <sub>2</sub> HPO <sub>4</sub>	8.0mg
Na <sub>2</sub> SiO <sub>3</sub>	4.0mg
NaF	2.4mg
NH <sub>4</sub> NO <sub>3</sub>	1.6mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution	0.5mL

pH 6.0 ± 0.2 at 25°C

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	1.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Neutralize to pH 7.0 with sterile HCl.

**Preparation of Medium:** Add components, except sulfur and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter through normal filter paper. An iron sediment will collect in the filter. Gently heat while stirring and bring to boiling. Boil for 5 min. Cool under an anaerobic gas mixture of N<sub>2</sub>. Adjust pH to 6.0. Distribute the medium into Hungate tubes or serum bottles containing finely divided sulfur (0.5% w/v). Seal the tubes or bottles under the same anaerobic gas used



when cooling the medium. Sterilize the medium at 100°C for 3 hr on 3 consecutive days. Reduce the medium by adding 10% neutralized Na<sub>2</sub>S·9H<sub>2</sub>O solution to a final concentration of 0.05%. The medium should not give a heavy black precipitate. If it does the iron sediment was not adequately removed by filtering in the initial stages and the medium should be made again, making sure that the iron is removed by filtering.

**Use:** For the cultivation, and maintenance of *Thermococcus aegaeus*.

### *Marinithermus hydrothermalis* Medium (DSMZ Medium 973)

#### Composition per liter:

NaCl	30.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	4.18g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.4g
Yeast extract	1.0g
Tryptone	1.0g
KCl	0.33g
NH <sub>4</sub> Cl	0.25g
K <sub>2</sub> HPO <sub>4</sub>	0.14g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	10.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.5mg
Na <sub>2</sub> Se <sub>3</sub> ·5H <sub>2</sub> O	0.5mg
Trace elements solution	10.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Marinithermus hydrothermalis*.

### *Marinitoga* Medium (DSMZ Medium 904)

#### Composition per 1045mL:

Sea salts	30.0g
PIPES	6.0g
Yeast extract	1.0g
Tryptone	1.0g
Resazurin	0.5mg
Glucose solution	25.0mL

Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
L-Cysteine solution	10.0mL
pH 7.0 ± 0.2 at 25°C	

#### Glucose Solution:

##### Composition per 25.0mL:

Sucrose	2.5g
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**Preparation of Glucose Solution:** Add sucrose to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### L-Cysteine Solution:

##### Composition per 10.0mL:

L-Cysteine·HCl·H <sub>2</sub> O	0.5g
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**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under parge with 100% N<sub>2</sub>. Add components, except glucose solution, L-Cysteine·HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring the volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter, 50.0mL glucose solution, 10.0mL L-Cysteine·HCl·H<sub>2</sub>O solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Marinitoga camini* and *Caloranaerobacter azorensis*.

### *Marinitoga piezophila* Medium (DSMZ Medium 945)

#### Composition per liter:

NaCl	30.0g
Yeast extract	5.0g
Trypticase	5.0g
MES	1.95g
NH <sub>4</sub> Cl	1.0g
Na-acetate	0.83g
K <sub>2</sub> HPO <sub>4</sub>	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
KCl	0.1g
Resazurin	0.5mg
Maltose solution	100.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Cysteine solution	10.0mL
pH 6.0 ± 0.2 at 25°C	

#### Maltose Solution:

##### Composition per 100.0mL:

Maltose	4.96g
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**Preparation of Maltose Solution:** Add maltose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Cysteine Solution:****Composition** per 10.0mL:L-Cysteine-HCl·H<sub>2</sub>O ..... 0.3g

**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add components, except maltose solution, vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution to 880.0mL distilled/deionized water. Mix thoroughly. Sparge for 30 min with 100% N<sub>2</sub>. Adjust pH to 6.0 with concentrated NaOH. Distribute under 100% N<sub>2</sub> into anaerobic tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 100.0mL sterile maltose solution, 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 10.0mL sterile cysteine solution per liter medium. Mix thoroughly.

**Use:** For the cultivation of *Marinitoga piezophila*.

***Marinobacter* Medium  
(DSMZ Medium 941)**

**Composition** per liter:

NaCl ..... 6.0g  
 NH<sub>4</sub>Cl ..... 1.0g  
 Na-acetate ..... 1.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
 KCl ..... 0.1g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.1g  
 Peptone ..... 0.1g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.04g  
 Trace elements solution SL-7 ..... 1.0mL  
 Vitamin solution, concentrated ..... 1.0mL

pH 7.2 ± 0.2 at 25°C

**Trace Elements Solution SL-7:****Composition** per liter:

FeCl<sub>2</sub>·7H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 62.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 17.0mg  
 HCl (25% solution) ..... 6.5mL

**Preparation of Trace Elements Solution SL-7:** Add FeCl<sub>2</sub>·7H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution, Concentrated:****Composition** per 100mL:

Pyridoxine-HCl ..... 10.0mg  
 Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Nicotinic acid ..... 5.0mg

D-Ca-pantothenate ..... 5.0mg  
 p-Aminobenzoic acid ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution, Concentrated:**

Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Marionobacter* sp.

***Marinococcus albus* Agar  
(LMG Medium 212)**

**Composition** per liter:

NaCl ..... 81.0g  
 Agar ..... 15.0g  
 Yeast ..... 10.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 9.6g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 7.0g  
 Proteose peptone ..... 5.0g  
 KCl ..... 2.0g  
 Glucose ..... 1.0g  
 CaCl<sub>2</sub> ..... 0.36g  
 NaB ..... 226.0mg  
 NaHCO<sub>3</sub> ..... 60.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Marinococcus albus*.

***Marinomonas vaga* Medium  
(DSMZ Medium 617)**

**Composition** per liter:

NaCl ..... 30.0g  
 Agar ..... 15.0g  
 Beef extract ..... 10.0g  
 Peptone ..... 10.0g

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Marinomonas communis*=*Alteromonas communis* and *Marinomonas vaga*=*Alteromonas vaga*.

**MB Medium  
(DSMZ Medium 924)**

**Composition** per liter:

NaCl ..... 10.0g  
 NaHCO<sub>3</sub> ..... 4.0g  
 Yeast extract ..... 2.0g  
 Trypticase ..... 2.0g  
 NH<sub>4</sub>Cl ..... 1.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 1.0g  
 KCl ..... 0.5g

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.4g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
Resazurin.....	0.5mg
Sodium formate solution.....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Cysteine-HCl·H <sub>2</sub> O solution.....	10.0mL
Vitamin solution.....	10.0mL
Trace elements solution.....	10.0mL
Selenite-tungstate solution.....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O.....	0.25g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Sodium Formate Solution:****Composition per 50.0mL:**

Na-formate.....	6.8g
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**Preparation of Sodium Formate Solution:** Add sodium formate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Selenite-Tungstate Solution:****Composition per liter:**

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except sodium formate solution, NaHCO<sub>3</sub>, cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add solid NaHCO<sub>3</sub>. Mix thoroughly. Adjust pH to 6.8–7.0. Distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter 50.0mL sterile sodium formate solution, 10.0mL of sterile cysteine solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. The final pH should be 7.2.

**Use:** For the cultivation of *Methanocalculus taiwanensis*, *Methanococcus voltae* (*Methanococcus voltaei*), and *Methanofollis aquaemaris*.

**MD 1 Medium****Composition per liter:**

Pancreatic digest of casein.....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
CaCl <sub>2</sub> .....	0.5g
Trace elements solution.....	1.0mL
Vitamin B <sub>12</sub> solution.....	1.0mL

**Trace Elements Solution:****Composition per liter:**

EDTA.....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> .....	0.02g
KBr.....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
BaCl <sub>2</sub> .....	5.0mg
LiCl.....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O.....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:****Composition per 10.0mL:**

Vitamin B <sub>12</sub> .....	5.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of myxobacteria.

### Me15% MH Medium (DSMZ Medium 582)

**Composition** per liter:

NaCl .....	121.5g
MgSO <sub>4</sub> .....	14.4g
MgCl <sub>2</sub> .....	10.5g
Yeast extract .....	10.0g
Proteose peptone no. 3 .....	5.0g
KCl .....	3.0g
Glucose .....	1.0g
CaCl <sub>2</sub> .....	0.54g
NaBr .....	0.039g
NaHCO <sub>3</sub> solution .....	10.0mL

pH 7.5 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	0.9g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Bacillus halophilus*.

### MED IIa

**Composition** per liter:

Tris buffer stock solution .....	10.0mL
CaCl <sub>2</sub> (5.0% solution) .....	10.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O (3.33% solution) .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Tris Buffer Stock Solution:**

**Composition** per 500.0mL:

Tris(hydroxymethyl)aminomethane·HCl .....	35.01g
Tris(hydroxymethyl)aminomethane .....	3.35g

**Preparation of Tris Buffer Stock Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 7.2.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Vampirovibrio chlorellavorus*.

### Medium A

**Composition** per liter:

D-Glucose .....	20.0g
Agar .....	20.0g
Yeast extract .....	10.0g
Biotin .....	1.0mg
Calcium pantothenate .....	1.0mg

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except biotin and calcium pantothenate, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat

and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Add biotin and calcium pantothenate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically add the sterile biotin and calcium pantothenate solution to the cooled sterile basal medium. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Zymomonas mobilis*.

### Medium 2A

**Composition** per liter:

Arginine .....	10.0g
NaCl .....	5.0g
Agar .....	4.0g
Peptone .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.3g
Phenol Red .....	0.01g

pH 7.2–7.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of *Pseudomonas* species based on their production of arginine dihydrolase activity.

### Medium AS4

**Composition** per liter:

Sucrose .....	80.0g
PPLO broth without Crystal Violet .....	500.0mL
Horse serum .....	200.0mL
Phenol Red (0.5% solution) .....	5.0mL

pH 7.2 ± 0.2 at 25°C

**PPLO Broth without Crystal Violet:**

**Composition** per 500.0mL:

Beef heart, solids from infusion .....	11.53g
Peptone .....	2.33g
NaCl .....	1.15g

**Source:** PPLO broth without Crystal Violet is available as a premixed powder from BD Diagnostic Systems.

**Preparation of PPLO Broth without Crystal Violet:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Beef heart for infusion may be substituted; 100.0g of beef heart for infusion is equivalent to 500.0g of fresh heart tissue.

**Preparation of Medium:** Add components, except horse serum, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Adjust pH to 7.2. Autoclave for 10 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 200.0mL of noninactivated, sterile horse serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma melliferum*.

### Medium for Ammonia Oxidizers

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.13g
K <sub>2</sub> HPO <sub>4</sub> .....	0.09g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
Chelated iron .....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.02mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0μg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enrichment of ammonia-oxidizing bacteria from soil.

#### Medium for Ammonia Oxidizers, Brackish

**Composition** per liter:

CaCO <sub>3</sub> .....	5.0g
NH <sub>4</sub> Cl .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.05g
Seawater .....	400.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enrichment of ammonia-oxidizing bacteria from brackish specimens.

#### Medium for Ammonia Oxidizers, Marine

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.32g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Chelated iron .....	0.13g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.02mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0μg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.0μg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0μg
Seawater .....	1.0L

**Preparation of Medium:** Combine components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enrichment of marine ammonia-oxidizing bacteria.

#### Medium for Ammonia-Oxidizing Bacteria

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	235.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	200.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	40.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	40.0mg
Iron-EDTA-Phenol Red solution .....	1.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	variable

#### Na<sub>2</sub>CO<sub>3</sub> Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Iron-EDTA-Phenol Red Solution:

**Composition** per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0mg
Sodium EDTA .....	50.0mg
Phenol Red .....	50.0mg

#### Preparation of Iron-EDTA-Phenol Red Solution:

Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Add enough sterile Na<sub>2</sub>CO<sub>3</sub> solution to turn the medium pale pink. During incubation and growth of bacteria, add additional sterile Na<sub>2</sub>CO<sub>3</sub> solution to restore the pale pink color. Growth is complete when no further color change is observed.

**Use:** For the cultivation of *Nitrosolobus multiformis* and *Nitrosomonas europaea*.

#### Medium B for Sulfate Reducers (Postgate's Medium B for Sulfate Reducers)

**Composition** per liter:

Sodium lactate .....	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
CaSO <sub>4</sub> .....	1.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Ascorbic acid .....	0.1g
Thioglycollic acid .....	0.1g

pH 7.0–7.5 at 25°C

**Preparation of Medium:** Add components, except ascorbic acid and thioglycollic acid, to tap water and bring volume to 1.0L. For marine bacteria, NaCl may be added or seawater used in place of tap water. Mix thoroughly. Adjust pH to 7.0–7.5. Thioglycolate and ascorbate should be added immediately prior to sterilization. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and maintenance of *Desulfovibrio* species and *Desulfotomaculum* species. This medium turns black as a result of H<sub>2</sub>S production due to bacterial growth.

#### Medium for *Bacillus schlegelii*

**Composition** per liter:

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.3g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaHCO <sub>3</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	10.0mg
Ferric ammonium citrate solution .....	20.0mL
Trace elements solution SL-6 .....	5.0mL

pH 6.8 ± 0.2 at 25°C

#### Ferric Ammonium Citrate Solution:

**Composition** per 20.0mL:

Ferric ammonium citrate .....	0.05g
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**Preparation of Ferric Ammonium Citrate Solution:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-6:

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except ferric ammonium citrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile ferric ammonium citrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the chemolithotrophic growth of *Bacillus schlegelii*.

#### Medium for *Bacillus stearothermophilus*

**Composition** per 1001.0mL:

NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Yeast extract .....	0.2g
Casamino acids .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
Phenol solution .....	100.0mL
Trace elements solution SL-4 .....	1.0mL

pH 7.4 ± 0.2 at 25°C

#### Phenol Solution:

**Composition** per 100.0mL:

Phenol .....	0.47g
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**Preparation of Phenol Solution:** Add phenol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution SL-4:

**Composition** per liter:

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

#### Trace Elements Solution SL-6:

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except phenol solution and trace elements solution SL-4, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of sterile phenol solution and 1.0mL of sterile trace elements solution SL-4. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Bacillus stearothermophilus*.

#### Medium with Biphenyl (DSMZ Medium 457d)

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
Biphenyl .....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements solution SL-4 .....	10.0mL

pH 6.9 ± 0.2 at 25°C

#### Trace Elements Solution SL-4:

**Composition** per liter:

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

#### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Biphenyl Solution:

**Composition** per liter:

Biphenyl .....	10.0g
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**Preparation of Biphenyl Solution:** Add biphenyl to 1.0L ethanol. Mix thoroughly. Filter sterilize using a cellulose filter membrane.

**Preparation of Medium:** Add components, except biphenyl solution, to 1.0L distilled/deionized water. Adjust pH to 6.9. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Add an aliquot of the biphenyl solution to a sterile flask so that the final concentration will be 0.25g/L biphenyl, and let the ethanol evaporate. Aseptically add sterile medium to the crystal-layered flask.

**Use:** For the cultivation of biphenyl utilizing bacteria.

#### Medium C for Sulfate Reducers (Postgate's Medium C for Sulfate Reducers)

**Composition** per liter:

Sodium lactate .....	6.0g
Na <sub>2</sub> SO <sub>4</sub> .....	4.5g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Sodium citrate·2H <sub>2</sub> O .....	0.3g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.06g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.004g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. For marine bacteria, NaCl may be added or seawater used in place of distilled/deionized water. Mix thoroughly. Adjust pH to 7.5.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For detection, culturing and storage of *Desulfovibrio* species and many *Desulfotomaculum* species. This medium should be used when a clear culture medium is desired such as for chemostat culture. This medium may be cloudy after sterilization but usually clears on cooling. It turns black as a result of H<sub>2</sub>S production due to bacterial growth.

#### Medium for Carbon Monoxide Oxidizers

##### Composition per liter:

Agar .....	12.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	4.5g
NH <sub>4</sub> Cl .....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.03g
Ferric ammonium citrate .....	0.018g
Trace elements solution SL-6 .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

##### Trace Elements Solution SL-6:

##### Composition per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes. After inoculation, incubate in an atmosphere of 80% CO + 10% O<sub>2</sub> + 10% N<sub>2</sub>.

**Use:** For the chemoautotrophic cultivation and maintenance of *Alcaligenes* species, *Pseudomonas carboxydohydrogena*, and other *Pseudomonas* species.

#### Medium for Chlorate Respirers (DSMZ Medium 908)

##### Composition per liter:

Solution A .....	1.0L
Solution B .....	10.0mL
Solution C .....	10.0mL
Vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

##### Solution A:

##### Composition per liter:

NaHCO <sub>3</sub> .....	2.5g
Na-Acetate .....	1.36g
NaClO <sub>3</sub> .....	1.0g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.6g
NH <sub>4</sub> Cl .....	0.25g
KCl .....	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

##### Solution B:

##### Composition per 10mL:

MgSO <sub>4</sub> .....	30.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

##### Solution C:

Na <sub>2</sub> MoO <sub>4</sub> .....	25.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	25.0mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

##### Vitamin Solution:

##### Composition per liter:

Vitamin B <sub>12</sub> .....	50.0mg
Pantothenic acid .....	50.0mg
Riboflavin .....	50.0mg
Alpha-lipoic acid .....	50.0mg
p-aminobenzoic acid .....	50.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	50.0mg
Nicotinic acid .....	25.0mg
Nicotine amide .....	25.0mg
Biotin .....	20.0mg
Folic acid .....	20.0mg
Pyridoxamine-HCl .....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

##### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

##### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Aseptically and anaerobically combine 1000.0mL solution A, 10.0mL solution B, and 10.0mL solution C. Aseptically and anaerobically add 5.0mL vitamin solution and 1.0mL trace elements solution SL-10. Mix thoroughly. The pH should be 7.2.

**Use:** For the cultivation of *Dechloromonas agitata* and *Azospira oryzae*.

#### Medium with Chloroacrylic Acid (DSMZ Medium 457c)

##### Composition per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Chloroacrylic acid solution .....	20.0mL
Trace elements solution SL-4 .....	10.0mL
pH 6.9 ± 0.2 at 25°C	

#### Trace Elements Solution SL-4:

##### Composition per liter:

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

#### Trace Elements Solution SL-6:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Chloroacrylic Acid Solution:

##### Composition per liter:

3-Chloroacrylic acid .....	4.0g
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**Preparation of Chloroacrylic Acid Solution:** Add 3-Chloroacrylic acid to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Filter sterilize.

**Preparation of Medium:** Add components, except chloroacrylic acid solution, to 1.0L distilled/deionized water. Adjust pH to 6.9. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 20.0mL sterile chloroacrylic acid solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of chloroacrylic acid-utilizing *Burkholderia* sp. (*Burkholderia cepacia*), *Rhodococcus erythropolis* (*Arthrobacter picolinophilus*, and *Nocardia* spp.

### Medium for *Chlorobium ferrooxidans* (DSMZ Medium 29a)

##### Composition per 5.0L:

Solution A .....	4.0L
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	30.0mL
Solution C .....	5.0mL
Solution D .....	5.0mL

pH 6.8 at 25°C

#### Solution A:

##### Composition per 4.0L:

MgSO <sub>4</sub> .....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
KCl .....	1.7g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g
Na-acetate .....	0.82g

**Preparation of Solution A:** Add components to 4.0L distilled water. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C in 5-liter special bottle or flask with

four openings at the top, together with a teflon-coated magnetic bar. In this 5-liter bottle, two openings are for tubes in the central, silicon rubber stopper; one is a short, gas-inlet tube with a sterile cotton filter; and the other is an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps; one of these openings is for the addition of sterile solutions and the other serves as a gas outlet. After autoclaving, cool solution A to room temperature under a N<sub>2</sub> atmosphere with a positive pressure of 0.05–0.1 atm (a manometer for low pressure will be required). Saturate the cold medium with CO<sub>2</sub> by magnetic stirring for 30 min under a CO<sub>2</sub> atmosphere of 0.05–0.1 atm.

#### Solution B:

Distilled water .....	860.0mL
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**Preparation of Solution B:** Autoclave distilled water for 15 min at 15 psi pressure–121°C in a cotton-stoppered Erlenmeyer flask. Cool to room temperature under an atmosphere of N<sub>2</sub> in an anaerobic jar.

#### Solution C:

##### Composition per 100.0mL:

Vitamin B <sub>12</sub> .....	2.0mg
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**Preparation of Solution C:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

#### Solution D:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

**Preparation of Solution D:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	4.2g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution F:

##### Composition per 100.0mL:

FeSO <sub>4</sub> .....	25.0g
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**Preparation of Solution F:** Add FeSO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

**Preparation of Medium:** Add solutions B, C, D, and E to solution A through one of the screw-cap openings against a stream of either N<sub>2</sub> gas or better, a mixture of 95% N<sub>2</sub> and



5% CO<sub>2</sub> while the medium is magnetically stirred. Adjust the pH of the medium with sterile HCl or Na<sub>2</sub>CO<sub>3</sub> solution (2 M solutions) to pH 6.8. Distribute the medium aseptically through the medium outlet tube into sterile, 100mL bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05 - 0.1 atm) of the N<sub>2</sub>/CO<sub>2</sub> gas mixture. Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-cap bottles can be stored for several weeks or months in the dark.

**Use:** For the cultivation of *Chlorobium ferrooxidans*.

#### Medium D4

**Composition** per liter:

Agar .....	15.0g
Sucrose.....	10.0g
NH <sub>4</sub> Cl.....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> , anhydrous .....	2.3g
Pancreatic digest of casein.....	1.0g
Sodium dodecyl sulfate.....	0.6g
Glycerol .....	10.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation and cultivation of *Pseudomonas syringae*.

#### Medium D for Sulfate Reducers

(Postgate's Medium D for Sulfate Reducers)

**Composition** per liter:

Sodium pyruvate.....	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.6g
NH <sub>4</sub> Cl.....	1.0g
Yeast extract.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.004g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Malate or fumarate may also be used as a carbon source. For marine bacteria, NaCl may be added or seawater used in place of distilled/deionized water. Mix thoroughly. Adjust pH to 7.5. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfovibrio* species and *Desulfotomaculum* species that can grow in the absence of sulfate.

#### Medium D for Sulfate Reducers

(Postgate's Medium D for Sulfate Reducers)

**Composition** per liter:

MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.6g
Choline chloride.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
Yeast extract.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.004g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Malate or fumarate may also be used as a carbon source. For marine bacteria, NaCl may be added or seawater used in place of

distilled/deionized water. Mix thoroughly. Adjust pH to 7.5. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Desulfovibrio* species and *Desulfotomaculum* species that can grow in the absence of sulfate.

#### Medium D for *Thermus*

**Composition** per liter:

Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
NaNO <sub>3</sub> .....	0.7g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.1g
Nitrilotriacetic acid.....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.06g
NaCl .....	8.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	2.2mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5mg
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
FeCl <sub>3</sub> .....	0.28mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CuSO <sub>4</sub> .....	0.02mg

pH 8.2 ± 0.2 at 25°C

**Preparation of Medium:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 8.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thermus* species.

#### Medium D for *Thermus*, Modified

**Composition** per liter:

Agar.....	25.0g
Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
Salt solution.....	100.0mL

pH 8.2 ± 0.2 at 25°C

#### Salt Solution:

**Composition** per liter:

NaNO <sub>3</sub> .....	6.89g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	2.8g
KNO <sub>3</sub> .....	1.03g
Nitrilotriacetic acid.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.6g
NaCl .....	0.08g
FeCl <sub>3</sub> ·6H <sub>2</sub> O solution.....	10.0mL
Trace elements solution.....	10.0mL

#### FeCl<sub>3</sub>·6H<sub>2</sub>O Solution:

**Composition** per 100.0mL:

FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	47.0mg
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**Preparation of FeCl<sub>3</sub>·6H<sub>2</sub>O Solution:** Add 47.0mg of FeCl<sub>3</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Trace Elements Solution:

**Composition** per liter:

MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	1.7g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	46.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	25.0mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	25.0mg
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Salt Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Re-adjust pH to 8.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 8.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Thermus aquaticus*.

#### Medium for *Ectothiorhodospira*

**Composition** per 1001.0mL:

Basal medium .....	800.0mL
Solution C .....	200.0mL
Vitamin solution B .....	1.0mL

#### Basal Medium:

**Composition** per 800.0mL:

NaCl .....	180.0g
Na <sub>2</sub> SO <sub>4</sub> .....	20.0g
Na <sub>2</sub> CO <sub>3</sub> .....	6.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
Sodium succinate .....	1.0g
NH <sub>4</sub> Cl .....	0.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Yeast extract .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.05g
Trace elements solution A .....	1.0mL

pH 8.5 ± 0.2 at 25°C

#### Trace Elements Solution A:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	500.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	250.0mg
ZnCl <sub>2</sub> .....	100.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution A:** Add components to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 3 with 1N HCl. Bring volume to 1.0L with distilled/deionized water.

**Preparation of Basal Solution:** Add components to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Adjust pH to 8.5. Distribute into screw-capped bottles. Autoclave for 15 min at 14 psi pressure–120°C.

#### Vitamin Solution B:

**Composition** per 100.0mL:

Nicotinamide .....	35.0mg
Thiamine dichloride .....	30.0mg
<i>p</i> -Aminobenzoic acid .....	20.0mg
Biotin .....	10.0mg
Calcium DL-pantothenate .....	10.0mg

Pyridoxal·HCl .....	10.0mg
Vitamin B <sub>12</sub> .....	5.0mg

**Preparation of Vitamin Solution B:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Solution C:

**Composition** per 200.0mL:

NaHCO <sub>3</sub> .....	14.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 800.0mL of sterile basal solution, aseptically add 200.0mL of sterile solution C and 1.0mL of sterile vitamin solution B. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Ectothiorhodospira halochloris*.

#### Medium for *Erythrobacter longus* (DSMZ Medium 695)

**Composition** per liter:

Peptone .....	2.0g
Soytone .....	1.0g
Yeast extract .....	1.0g
Proteose peptone no.3 .....	1.0g
Ferric citrate solution .....	2.0mL
Artificial seawater .....	700.0mL

pH 7.5 ± 0.2 at 25°C

#### Artificial Seawater:

**Composition** per liter:

NaCl .....	23.477g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.981g
Na <sub>2</sub> SO <sub>4</sub> .....	3.917g
CaCl <sub>2</sub> .....	1.12g
KCl .....	664.0mg
NaHCO <sub>3</sub> .....	192.0mg
H <sub>3</sub> BO <sub>3</sub> .....	26.0mg
SrCl <sub>2</sub> .....	24.0mg
KBr .....	6.0mg
NaF .....	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Ferric Citrate Solution:

**Composition** per 10.0mL:

Ferric citrate .....	0.5g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except artificial seawater, to distilled/deionized water and bring volume to 300.0mL. Mix thoroughly. Adjust pH to 7.5. Aseptically add 700.0mL artificial seawater. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Erythrobacter longus*.

#### Medium E for Sulfate Reducers (Postgate's Medium E for Sulfate Reducers)

**Composition** per liter:

Agar .....	15.0g
Sodium lactate .....	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0g
NH <sub>4</sub> Cl .....	1.0g

Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.0g
Yeast extract.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Ascorbic acid.....	0.1g
Thioglycollic acid.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.004g

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except ascorbic acid and thioglycollic acid, to tap water and bring volume to 1.0L. For marine bacteria, NaCl may be added or seawater used in place of tap water. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.6. Thioglycolate and ascorbate should be added immediately prior to sterilization. Distribute into screw-capped tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and enumeration of *Desulfovibrio* species and *Desulfotomaculum* species as black colonies in deep agar cultures. Also used for the isolation of pure cultures of *Desulfovibrio* species and *Desulfotomaculum* species.

### Medium F

#### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.15g
KCl.....	0.05g
KH <sub>2</sub> PO <sub>4</sub> .....	0.05g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution.....	10.0mL

pH 3.5 ± 0.2 at 25°C

#### FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
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**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add the FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except FeSO<sub>4</sub>·7H<sub>2</sub>O solution, to tap water and bring volume to 990.0mL. Mix thoroughly. Gently heat until dissolved. Adjust pH to 3.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile FeSO<sub>4</sub>·7H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus* species.

### Medium F for Sulfate Reducers (Postgate's Medium F for Sulfate Reducers)

#### Composition per liter:

Agar.....	12.0g
Pancreatic digest of casein.....	10.0g
Sodium lactate.....	3.5g
Ferrous citrate.....	0.5g
Na <sub>2</sub> SO <sub>3</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Ascorbic acid.....	0.1g
Sodium thioglycolate.....	0.1g

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except ascorbic acid and thioglycollic acid, to tap water and bring volume to 1.0L. For marine bacteria, NaCl may be added or seawater used in place of tap water. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.1. Thioglycolate and ascorbate should be added immediately prior to steril-

ization. Distribute into screw-capped tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For isolation and cultivation of *Desulfotomaculum nigrificans*, *Desulfovibrio* species, and other *Desulfotomaculum* species especially in food. These bacteria form black colonies in deep agar cultures.

### Medium with Fluoranthene (DSMZ Medium 457b)

#### Composition per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Twen 80.....	0.2g
Fluoranthene.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
Trace elements solution SL-4.....	10.0mL

pH 6.9 ± 0.2 at 25°C

#### Trace Elements Solution SL-4:

##### Composition per liter:

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

#### Trace Elements Solution SL-6:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Fluoranthene Solution:

##### Composition per liter:

Fluoranthene.....	2.0g
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**Preparation of Fluoranthene Solution:** Add fluoranthene to 1.0L acetone. Mix thoroughly. Filter sterilize using a cellulose filter membrane.

**Preparation of Medium:** Add components, except fluoranthene solution, to 1.0L distilled/deionized water. Adjust pH to 6.9. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Add an aliquot of the fluoranthene solution to a sterile flask so that the final concentration will be 0.1g/L fluoranthene, and let the acetone evaporate. Aseptically add sterile medium to the crystal-layered flask.

**Use:** For the cultivation of fluoranthene-utilizing *Pseudomonas frederiksbergensis*, *Sphingomonas* sp. (*Pseudomonas paucimobilis*), and other bacteria.

### Medium for Freshwater Flexibacteria

#### Composition per 1002.0mL:

Casamino acids.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
Tris (hydroxymethyl) amino methane.....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
KNO <sub>3</sub> .....	0.1g

Sodium glycerophosphate.....	0.1g
Thiamine.....	1.0mg
Cobalamine.....	1.0µg
Glucose solution.....	1.0mL
Trace elements solution.....	1.0mL

pH  $7.5 \pm 0.2$  at 25°C**Glucose Solution:****Composition per 100.0mL:**

Glucose.....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

ZnCl <sub>2</sub> .....	20.8g
H <sub>3</sub> BO <sub>3</sub> .....	2.85g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8g
Sodium tartrate.....	1.77g
FeSO <sub>4</sub> .....	1.36g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	40.4mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	26.9mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	25.2mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution and trace elements solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mL of sterile glucose solution and 1.0mL of sterile trace elements solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Cytophaga psychrophila*, *Fleotobacillus major*, *Flexibacter aurantiacus*, *Flexibacter aurantiacus*, *Flexibacter elegans*, *Flexibacter flexilis*, *Flexibacter roseolus*, *Flexibacter ruber*, *Flexibacter sancti*, and *Herpetosiphon geysericola*.

**Medium G for Sulfate Reducers****(Postgate's Medium G for Sulfate Reducers)****Composition per 1015.2mL:**

Solution 1.....	970.0mL
Solution 4.....	30.0mL
Solution 8A, 8B, 8C, 8D or 8E.....	10.0mL
Solution 5.....	3.0mL
Solution 2.....	1.0mL
Solution 3.....	1.0mL
Solution 6.....	0.1mL
Solution 7.....	0.1mL

pH  $7.2 \pm 0.2$  at 25°C**Solution 1:****Composition per 970.0mL:**

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl.....	1.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
KCl.....	0.3g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 7.2 with 2N HCl. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 2:****Composition per 10.0mL:**

NaOH.....	5.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	0.03mg

**Preparation of Solution 2:** Add NaOH and Na<sub>2</sub>SeO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 3:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.12g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.025g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.015g

**Preparation of Solution 3:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 4:****Composition per 30.0mL:**

NaHCO <sub>3</sub> .....	2.55g
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**Preparation of Solution 4:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Gas with 100% CO<sub>2</sub> for 10–15 min. Filter sterilize.

**Solution 5:****Composition per 3.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.36g
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**Preparation of Solution 5:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 3.0mL. Mix thoroughly. Gas with 100% N<sub>2</sub> for 5–10 min. Cap tube with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 6:****Composition per 100.0mL:**

Thiamine·HCl.....	0.01g
Cyanocobalamin.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Biotin.....	1.0mg

**Preparation of Solution 6:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution 7:****Composition per 100.0mL:**

Succinic acid.....	0.6g
Isobutyric acid.....	0.5g
Valeric acid.....	0.5g
2-Methylbutyric acid.....	0.5g
3-Methylbutyric acid.....	0.5g
Caproic acid.....	0.2g

**Preparation of Solution 7:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 9.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 8A:****Composition per 100.0mL:**

Sodium acetate·3H <sub>2</sub> O.....	20.0g
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**Solution 8B:****Composition** per 100.0mL:

Propionic acid ..... 7.0g

**Solution 8C:****Composition** per 100.0mL:*n*-Butyric acid ..... 8.0g**Solution 8D:****Composition** per 100.0mL:

Benzoic acid ..... 5.0g

**Solution 8E:****Composition** per 100.0mL:*n*-Palmitic acid ..... 5.0g

**Preparation of Solutions 8A–E:** Add the appropriate amount of component to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 9.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** To 970.0mL of cooled, sterile solution 1, aseptically add 1.0mL of sterile solution 2, 1.0mL of sterile solution 3, 30.0mL of sterile solution 4, 3.0mL of sterile solution 5, 0.1mL of sterile solution 6, 0.1mL of sterile solution 7, and 10.0mL of sterile solution 8A, 8B, 8C, 8D, or 8E. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Desulfovibrio baarsii*, *Desulfovibrio sapovorans*, *Desulfobacter* species, *Desulfonema* species, *Desulfobulbus* species, and *Desulfotomaculum acetoxidans*.

**Medium for Halophilic Bacilli****Composition** per liter:

NaCl ..... 100.0g

Casamino acids ..... 10.0g

Yeast extract ..... 10.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of halophilic *Bacillus* species.

**Medium for Hydrocarbon-Degrading Bacteria****Composition** per 1020.0mL:NH<sub>4</sub>Cl ..... 0.5gMgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g

NaCl ..... 0.4g

Hydrocarbon ..... 20.0mL

KH<sub>2</sub>PO<sub>4</sub> solution ..... 0.5mLNa<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O solution ..... 0.5mL**KH<sub>2</sub>PO<sub>4</sub> Solution:****Composition** per 100.0mL:KH<sub>2</sub>PO<sub>4</sub> ..... 10.0g

**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Na<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O Solution:****Composition** per 100.0mL:Na<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O ..... 10.0g

**Preparation of Na<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O Solution:** Add 10.0g of Na<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components—except hydrocarbon, KH<sub>2</sub>PO<sub>4</sub> solution, and Na<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 0.5mL of sterile KH<sub>2</sub>PO<sub>4</sub> solution and 0.5mL of the sterile Na<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes in 10.0mL volumes. Add 0.2mL of sterile hydrocarbon to each tube.

**Use:** For the cultivation and enumeration of hydrocarbon-degrading bacteria in fresh water.

**Medium for Hydrocarbon-Degrading Bacteria (Naphthalene Mineral Salts Medium)****Composition** per liter:K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0gMgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.3gCaCl<sub>2</sub> ..... 0.1gFeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.02g

Naphthalene ..... 2.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except naphthalene, to distilled/deionized water and bring volume to 998.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 2.0mL of sterile naphthalene to 20.0mL of sterile basal salts. Ultrasonically homogenize the solution. Add the naphthalene–basal salts homogenate back to the remainder of the sterile basal salts medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and enrichment of hydrocarbon-degrading bacteria.

**Medium M71****Composition** per liter:

Agar ..... 20.0g

Peptone ..... 10.0g

Glucose ..... 5.0g

H<sub>3</sub>BO<sub>3</sub> ..... 1.0g

Pancreatic digest of casein ..... 1.0g

Cycloheximide ..... 0.05g

2,3,5-Triphenyltetrazolium-HCl solution ..... 10.0mL

**2,3,5-Triphenyltetrazolium-HCl Solution:****Composition** per 10.0mL:

2,3,5-Triphenyltetrazolium-HCl ..... 0.05g

**Preparation of 2,3,5-Triphenyltetrazolium-HCl Solution:** Add 2,3,5-triphenyltetrazolium-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except 2,3,5-triphenyltetrazolium-HCl solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile 2,3,5-triphenyltetrazolium-HCl solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the selective isolation and cultivation of *Pseudomonas syringae*.

**Medium 523M****Composition** per liter:

Agar ..... 15.0g

Sucrose ..... 10.0g

Casamino acids .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Yeast extract .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Clavibacter toxicus*.

#### Medium for Marine Flexibacteria

**Composition** per 1001.0mL:

Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
Tris (hydroxymethyl) amino methane .....	1.0g
KNO <sub>3</sub> .....	0.5g
Sodium glycerophosphate .....	0.1g
Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Solution:

**Composition** per liter:

ZnCl <sub>2</sub> .....	20.8g
H <sub>3</sub> BO <sub>3</sub> .....	2.85g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
Sodium tartrate .....	1.77g
FeSO <sub>4</sub> .....	1.36g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	40.4mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	26.9mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	25.2mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except trace elements solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mL of sterile trace elements solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Cytophaga aprica*, *Cytophaga diffluens*, *Cytophaga johnsonae*, *Cytophaga lytica*, *Cytophaga* species, *Flexibacter aggregans*, *Flexibacter aurantia*, *Flexibacter litoralis*, *Flexibacter tractuosus*, *Flexithrix dorotheae*, *Herpetosiphon cohaerens*, *Herpetosiphon nigricans*, *Herpetosiphon persicus*, *Microscilla arenaria*, *Microscilla furvescens*, *Microscilla marina*, *Microscilla sericea*, and *Saprospira grandis*.

#### Medium for Marine Methylotrophs (DSMZ Medium 750)

**Composition** per liter:

NaCl .....	25.0g
Agar .....	20.0g
Peptone .....	10.0g
Beef extract .....	7.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Methanol .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL filter sterilized methanol.

Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylophaga marina* and *Methylophaga thalassica*.

#### Medium N for Sulfate Reducers

(Postgate's Medium N for Sulfate Reducers)

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	7.0g
Sodium lactate .....	6.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Sodium citrate·2H <sub>2</sub> O .....	0.3g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.06g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.06g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. For marine bacteria, NaCl may be added or seawater used in place of distilled/deionized water. Mix thoroughly. Adjust pH to 7.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the detection, culturing, and storage of *Desulfovibrio* species and many *Desulfotomaculum* species. This medium should be used when a clear culture medium is desired such as for chemostat culture. This medium may be cloudy after sterilization but usually clears on cooling. It turns black as a result of H<sub>2</sub>S production due to bacterial growth.

#### Medium for Nitrite Oxidizers

**Composition** per liter:

KHCO <sub>3</sub> .....	1.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
KNO <sub>2</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enrichment of nitrate-oxidizing bacteria.

#### Medium for Nitrite Oxidizers, Marine

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NaNO <sub>2</sub> .....	0.07g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	6.0mg
K <sub>2</sub> HPO <sub>4</sub> .....	1.74mg
Chelated iron .....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	66.0μg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0μg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	30.0μg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	6.0μg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.6μg
Seawater .....	700.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enrichment of marine nitrate-oxidizing bacteria.

### Medium for Osmophilic Fungi (M 40 Y)

**Composition** per liter:

Sucrose.....	400.0g
Agar .....	20.0g
Malt extract .....	20.0g
Yeast extract.....	5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of osmophilic fungi.

### Medium with Phenanthrene (DSMZ Medium 457b)

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
Phenanthrene.....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Tween 80.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
Trace elements solution SL-4 .....	10.0mL

pH 6.9 ± 0.2 at 25°C

### Trace Elements Solution SL-4:

**Composition** per liter:

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

### Phenanthrene Solution:

**Composition** per liter:

Phenanthrene.....	2.0g
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**Preparation of Phenanthrene Solution:** Add phenanthrene to 1.0L acetone. Mix thoroughly. Filter sterilize using a cellulose filter membrane.

**Preparation of Medium:** Add components, except phenanthrene solution, to 1.0L distilled/deionized water. Adjust pH to 6.9. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Add an aliquot of the phenanthrene solution to a sterile flask so that the final concentration will be 0.1g/L phenanthrene, and let the acetone

evaporate. Aseptically add sterile medium to the crystal-layered flask.

**Use:** For the cultivation of phenanthrene-utilizing *Sphingomonas* sp. (*Pseudomonas paucimobilis*), *Pseudomonas frederiksbergensis*, and other bacteria.

### Medium for *Prosthecomicrobium* and *Ancalomicrobium*

**Composition** per liter:

Agar.....	15.0g
Peptone .....	0.1g
Hutner's modified salts solution.....	20.0mL
Vitamin solution .....	10.0mL

### Hutner's Mineral Base:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	29.7g
Nitrilotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

**Preparation of Hutner's Mineral Base:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

### "Metals 44":

**Composition** per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA.....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl.....	0.01g
Calcium pantothenate .....	5.0mg
Nicotinamide .....	5.0mg
Riboflavin.....	5.0mg
Thiamine HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation of *Prosthecomicrobium* species and *Ancalomicrobium* species.

### Medium R

**Composition** per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	5.0g
KNO <sub>3</sub> .....	2.0g

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**KH<sub>2</sub>PO<sub>4</sub> Solution:****Composition** per 10.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**NaHCO<sub>3</sub> Solution:****Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:****Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg
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**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 10.0mg of FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except KH<sub>2</sub>PO<sub>4</sub> solution, NaHCO<sub>3</sub> solution, and FeSO<sub>4</sub>·7H<sub>2</sub>O solution, to tap water and bring volume to 970.0mL. Mix thoroughly. Gently heat until dissolved. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile KH<sub>2</sub>PO<sub>4</sub> solution, 10.0mL of NaHCO<sub>3</sub> solution, and 10.0mL of FeSO<sub>4</sub>·7H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus denitrificans*.

**Medium S****Composition** per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
MgSO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> .....	0.25g
FeSO <sub>4</sub> .....	0.01g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus* species.

**Medium SP 4****Composition** per liter:

Pancreatic digest of casein .....	11.0g
Peptone .....	5.3g
Glucose .....	5.0g
NaCl .....	0.875g
Beef extract .....	0.525g
Yeast extract .....	0.525g
Beef heart, solids from infusion .....	0.35g
Fetal bovine serum, heat inactivated .....	170.0mL
Yeast extract solution .....	100.0mL
CMRL 1066, 10× solution .....	50.0mL
Fresh yeast extract solution .....	35.0mL

Phenol Red solution .....	20.0mL
Penicillin solution .....	10.0mL
pH 7.6 ± 0.2 at 25°C	

**Yeast Extract Solution:****Composition** per 100.0mL:

Yeast extract .....	2.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**CMRL 1066, 10X Solution:****Composition** per liter:

NaCl .....	6.8g
NaHCO <sub>3</sub> .....	2.2g
D-Glucose .....	1.0g
KCl .....	0.4g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.26g
CaCl <sub>2</sub> , anhydrous .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	0.14g
L-Glutamine .....	0.1g
Sodium acetate·3H <sub>2</sub> O .....	0.083g
L-Glutamic acid .....	0.075g
L-Arginine·HCl .....	0.07g
L-Lysine·HCl .....	0.07g
L-Leucine .....	0.06g
Glycine .....	0.05g
Ascorbic acid .....	0.05g
L-Proline .....	0.04g
L-Tyrosine .....	0.04g
L-Aspartic acid .....	0.03g
L-Threonine .....	0.03g
L-Alanine .....	0.025g
L-Phenylalanine .....	0.025g
L-Serine .....	0.025g
L-Valine .....	0.025g
L-Cystine .....	0.02g
L-Histidine·HCl·H <sub>2</sub> O .....	0.02g
L-Isoleucine .....	0.02g
Phenol Red .....	0.02g
L-Methionine .....	0.015g
Deoxyadenosine .....	0.01g
Deoxycytidine .....	0.01g
Deoxyguanosine .....	0.01g
Glutathione, reduced .....	0.01g
Thymidine .....	0.01g
Hydroxy-L-proline .....	0.01g
L-Tryptophan .....	0.01g
Nicotinamide adenine dinucleotide .....	7.0mg
Tween™ 80 .....	5.0mg
Sodium glucuronate·H <sub>2</sub> O .....	4.2mg
Coenzyme A .....	2.5mg
Coccarboxylase .....	1.0mg
Flavin adenine dinucleotide .....	1.0mg
Nicotinamide adenine dinucleotide phosphate .....	1.0mg
Uridine triphosphate .....	1.0mg
Choline chloride .....	0.5mg
Cholesterol .....	0.2mg
5-Methyldeoxycytidine .....	0.1mg
Inositol .....	0.05mg
p-Aminobenzoic acid .....	0.05mg
Niacin .....	0.025mg
Niacinamide .....	0.025mg
Pyridoxine .....	0.025mg
Pyridoxal·HCl .....	0.025mg



Biotin .....	0.01mg
D-Calcium pantothenate .....	0.01mg
Folic acid .....	0.01mg
Riboflavin .....	0.01mg
Thiamine-HCl .....	0.01mg

**Source:** CMRL 1066, 10× medium is available as a premixed powder from BD Diagnostics.

**Preparation of CMRL 1066, 10X Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Filter sterilize.

#### **Fresh Yeast Extract Solution:**

**Composition per 100.0mL:**

Baker's yeast, live, pressed, starch-free .....	25.0g
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**Preparation of Fresh Yeast Extract Solution:** Add the live Baker's yeast to 100.0mL of distilled/deionized water. Autoclave for 90 min at 15 psi pressure–121°C. Allow to stand. Remove supernatant solution. Adjust pH to 6.6–6.8. Filter sterilize.

#### **Phenol Red Solution:**

**Composition per 100.0mL:**

Phenol Red .....	0.01g
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**Preparation of Phenol Red Solution:** Add Phenol Red to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Penicillin Solution:**

**Composition per 10.0mL:**

Penicillin .....	1,000,000U
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**Preparation of Penicillin Solution:** Add penicillin to distilled/deionized water and bring volume to 10.0mL. Filter sterilize.

**Preparation of Medium:** Add components—except fetal bovine serum, yeast extract solution, CMRL 1066, 10× solution, fresh yeast extract solution, Phenol Red solution, and penicillin solution—to distilled/deionized water and bring volume to 615.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 170.0mL of sterile fetal bovine serum, 100.0mL of sterile yeast extract solution, 50.0mL of sterile CMRL 1066, 10× solution, 35.0mL of sterile fresh yeast extract solution, 20.0mL of sterile Phenol Red solution, and 10.0mL of sterile penicillin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Spiroplasma* species from ticks.

### **Medium for Sulfate Reducers (Postgate's Medium for Sulfate Reducers) (ATCC Medium 1283)**

**Composition per liter:**

Part A .....	869.0mL
Part C .....	100.0mL
Part D .....	10.0mL
Part E .....	10.0mL
Part F .....	10.0mL
Part B .....	1.0mL

pH 7.7 ± 0.2 at 25°C

#### **Part A:**

**Composition per 869.0mL:**

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g

NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g

**Preparation of Part A:** Add components to distilled/deionized water and bring volume to 869.0mL. Mix thoroughly. Prepare and autoclave part A under 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Part B:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
HCl, 25% .....	10.0mL

**Preparation of Part B:** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to the HCl. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Part C:**

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Part C:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas with 90% N<sub>2</sub> + 10% CO<sub>2</sub> to remove residual O<sub>2</sub>.

#### **Part D:**

**Composition per 10.0mL:**

Sodium butyrate .....	0.7g
Sodium caproate .....	0.3g
Sodium octanoate .....	0.15g

**Preparation of Part D:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Part E:**

**Composition per 10.0mL:**

Yeast extract .....	1.0g
Thiamine-HCl .....	100.0μg
p-Aminobenzoic acid .....	40.0μg
D(+)-Biotin .....	10.0μg

**Preparation of Part E:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Part F:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Part F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** To 869.0mL of sterile cooled Part A, aseptically add the remaining sterile solutions in the following order: part B, part C, part D, part E, and part F. Mix thoroughly. Adjust pH to 7.7. Anaerobically distribute under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Desulfovibrio baarsii* and *Desulfovibrio sapovorans*.

#### Medium for Thermophilic Actinomycetes

##### Composition per liter:

Agar .....	20.0g
Soluble starch .....	10.0g
Maize extract .....	5.0g
NaCl .....	5.0g
Peptone .....	5.0g
CaCl <sub>2</sub> .....	0.5g

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of thermophilic actinomycetes.

#### Megasphaera Medium

##### Composition per liter:

Yeast extract .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.2g
KH <sub>2</sub> PO <sub>4</sub> .....	1.6g
Agar .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
Sodium thioglycolate .....	0.45g
CaCl <sub>2</sub> .....	0.2g
MgCl <sub>2</sub> .....	0.2g
Sodium lactate (60% solution) .....	16.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Megasphaera elsdenii*.

#### Melissococcus pluton Medium

##### Composition per liter:

Glucose .....	10.0g
Neopeptone .....	5.0g
Peptone .....	2.5g
Yeast extract .....	2.5g
Soluble starch .....	2.0g
Pancreatic digest of casein .....	2.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.25g
Phosphate buffer (1M, pH 6.7) .....	50.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks that have been flushed with 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Cap with butyl rubber stoppers. Place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Melissococcus pluton*.

#### Membrane Lauryl Sulfate Broth

##### Composition per liter:

Peptone .....	39.0g
Lactose .....	30.0g
Yeast extract .....	6.0g

Sodium lauryl sulfate .....	1.0g
Phenol Red .....	0.2g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enumeration of coliform organisms and *Escherichia coli* in water.

#### Metal Acetate Agar

##### Composition per liter:

Agar .....	15.0g
Sodium acetate .....	2.0g
Beijerinck's solution .....	50.0mL
Phosphate buffer solution .....	50.0mL
Trace elements solution .....	1.0mL

pH 6.8 ± 0.2 at 25°C

#### Beijerinck's Solution:

##### Composition per liter:

NH <sub>4</sub> Cl .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g

**Preparation of Beijerinck's Solution:** Add the CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Add the remaining components to distilled/deionized water and bring volume to 500.0mL in a separate flask. Combine the two solutions.

#### Phosphate Buffer Solution:

##### Composition per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	28.8g
KH <sub>2</sub> PO <sub>4</sub> .....	14.4g

**Preparation of Phosphate Buffer Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution:

##### Composition per liter:

EDTA .....	50.0g
H <sub>3</sub> BO <sub>3</sub> solution .....	200.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	100.0mL
CoCl <sub>2</sub> ·6H <sub>2</sub> O solution .....	50.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O solution .....	50.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	50.0mL
MnCl <sub>2</sub> ·4H <sub>2</sub> O solution .....	50.0mL
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O solution .....	50.0mL

#### H<sub>3</sub>BO<sub>3</sub> Solution:

##### Composition per 200.0mL:

H <sub>3</sub> BO <sub>3</sub> .....	11.4g
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**Preparation of H<sub>3</sub>BO<sub>3</sub> Solution:** Add H<sub>3</sub>BO<sub>3</sub> to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly.

#### ZnSO<sub>4</sub>·7H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	22.0g
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**Preparation of ZnSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add ZnSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### MnCl<sub>2</sub>·4H<sub>2</sub>O Solution:

##### Composition per 50.0mL:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.06g
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**Preparation of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  Solution:** Add 4.99g of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  Solution:**

**Composition per 50.0mL:**

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 4.99g

**Preparation of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  Solution:** Add 4.99g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  Solution:**

**Composition per 50.0mL:**

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 1.61g

**Preparation of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  Solution:** Add 1.61g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  Solution:**

**Composition per 50.0mL:**

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 1.57g

**Preparation of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  Solution:** Add 1.57g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  Solution:**

**Composition per 50.0mL:**

$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  ..... 1.1g

**Preparation of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  Solution:** Add  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly.

**Preparation of Trace Elements Solution:** Add EDTA to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until dissolved. Add 200.0mL of  $\text{H}_3\text{BO}_3$  solution, 100.0mL of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  solution, 50.0mL of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  solution, 50.0mL of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution, 50.0mL of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  solution, 50.0mL of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  solution, and 50.0mL of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  solution. Gently heat and bring to boiling. Cool to 70°C. Adjust pH to 6.8 with hot (70°C) 20% KOH solution. Add distilled/deionized water and bring volume to 1.0L. Allow solution to stand in a 2.0L cotton-stoppered flask at room temperature until the solution turns purple (approximately 2 weeks). Filter using two layers of Whatman #1 filter paper. Filter until clear.

**Preparation of Medium:** Add components, except phosphate buffer solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile phosphate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Chlamydomonas reinhardtii*.

***Metallogenium* Cultivation Broth**

**Composition per liter:**

Starch, hydrolyzed ..... 20.0g

$\text{MnCO}_3$  ..... 0.5g

**$\text{MnCO}_3$ :**

**Composition per 100.0mL:**

$\text{MnCl}_2$  ..... 20.0g

$\text{NaHCO}_3$  (25% solution) ..... 25.0mL

**Preparation of  $\text{MnCO}_3$ :** Add  $\text{MnCl}_2$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

Add  $\text{NaHCO}_3$  solution. Filter through Whatman #1 filter paper. Save the  $\text{MnCO}_3$  precipitate. Wash and store under distilled/deionized water.

**Preparation of Medium:** Hydrolyze starch with HCl.

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Metallogenium* species.

***Metallogenium* Isolation Agar**

**Composition per liter:**

Agar ..... 15.0g

Manganese acetate ..... 0.1g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Metallogenium* species.

***Metallogenium* Medium**

**Composition per liter:**

$\text{MnCO}_3$  ..... 2.0g

Starch, hydrolyzed ..... 1.0g

DNA ..... 0.01g

Catalase ..... 5.0mg

*Mycoplasma* broth base ..... 100.0mL

Yeast extract, ultrafiltrate ..... 100.0mL

Horse serum ..... 10.0mL

***Mycoplasma* Broth Base:**

**Composition per liter:**

Pancreatic digest of casein ..... 7.0g

$\text{NaCl}$  ..... 5.0g

Beef extract ..... 3.0g

Yeast extract ..... 3.0g

Beef heart, solids from infusion ..... 2.0g

**Preparation of *Mycoplasma* Broth Base:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**$\text{MnCO}_3$ :**

**Composition per 100.0mL:**

$\text{MnCl}_2$  ..... 20.0g

$\text{NaHCO}_3$  (25% solution) ..... 25.0mL

**Preparation of  $\text{MnCO}_3$ :** Add  $\text{MnCl}_2$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Add  $\text{NaHCO}_3$  solution. Filter through Whatman #1 filter paper. Save the  $\text{MnCO}_3$  precipitate. Wash and store under distilled/deionized water.

**Preparation of Medium:** Add  $\text{MnCO}_3$ , hydrolyzed starch, and DNA to 25.0mL of distilled/deionized water. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile *Mycoplasma* broth base, 100.0mL of ultrafiltrate of yeast extract, 10.0mL of horse serum, and 5.0mg of catalase. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Metallogenium* species.

***Metallosphaera* Medium**

**Composition per liter:**

$(\text{NH}_4)_2\text{SO}_4$  ..... 1.3g

Yeast extract ..... 1.0g

KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> B <sub>4</sub> ·10H <sub>2</sub> O.....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2.0 using 10N H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Metallosphaera sedula*.

### Methanobacillus Medium

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	9.0g
K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
NH <sub>4</sub> Cl.....	5.0g
MgCl <sub>2</sub> .....	1.0g
CaCl <sub>2</sub> .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01g
Ethanol.....	10.0mL

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Filter sterilize ethanol. Add components, except ethanol, to tap water and bring volume to 990.0mL. Mix thoroughly. Gently heat until dissolved. Autoclave for 20 min at 10psi pressure–115°C. Cool to 45°–50°C. Aseptically add sterile ethanol. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the selective isolation and cultivation of *Methanobacillus* species from mixed cultures.

### Methanobacteria Medium

**Composition** per liter:

Mineral solution 2.....	50.0mL
Sodium carbonate solution.....	50.0mL
Mineral solution 1.....	25.0mL
L-Cysteine-sulfide reducing agent.....	20.0mL
Wolfe's mineral solution.....	10.0mL
Vitamin solution.....	10.0mL
Resazurin (0.025% solution).....	4.0mL

pH 7.2 ± 0.2 at 25°C

### Mineral Solution 1:

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
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**Preparation of Medium:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Mineral Solution 2:

**Composition** per liter:

NaCl.....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.6g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Sodium Carbonate Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	8.0g
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**Preparation of Sodium Carbonate Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

### L-Cysteine-Sulfide Reducing Agent:

**Composition** per 20.0mL:

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g

### Preparation of L-Cysteine-Sulfide Reducing Agent:

Add L-cysteine·HCl·H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. In a separate tube, add Na<sub>2</sub>S·9H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. Gas both solutions with 100% N<sub>2</sub> and cap tubes. Autoclave both solutions for 15 min at 15 psi pressure–121°C using fast exhaust. Cool to 50°C. Aseptically combine the two solutions under 100% N<sub>2</sub>.

### Wolfe's Mineral Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitriloacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water and bring volume to 1.0L.

### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine·HCl.....	10.0mg
Thiamine·HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
Calcium pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution and L-cysteine-sulfide reducing agent, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically add the sterile vitamin solution and then the sterile L-cysteine-sulfide reducing agent. Adjust the pH to 7.2. Distribute aseptically and anaerobically into sterile tubes.

**Use:** For the cultivation and maintenance of *Acetogenium kivui*, *Methanobacterium formicicum*, *Methanobacterium thermoautotrophicum*, and *Methanobrevibacter arborophilicus*.

### **Methanobacteria Medium with Glucose and Yeast Extract**

#### **Composition per liter:**

Glucose .....	5.0g
Yeast extract .....	2.0g
Mineral solution 2 .....	50.0mL
Sodium carbonate solution .....	50.0mL
Mineral solution 1 .....	25.0mL
L-Cysteine-sulfide reducing agent .....	20.0mL
Wolfe's mineral solution .....	10.0mL
Vitamin solution .....	10.0mL
Resazurin (0.025% solution) .....	4.0mL

pH 7.2 ± 0.2 at 25°C

#### **Mineral Solution 1:**

##### **Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
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**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Mineral Solution 2:**

##### **Composition per liter:**

NaCl .....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.6g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Sodium Carbonate Solution:**

##### **Composition per 100.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	8.0g
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**Preparation of Sodium Carbonate Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **L-Cysteine-Sulfide Reducing Agent:**

##### **Composition per 20.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g

**Preparation of L-Cysteine-Sulfide Reducing Agent:** Add L-cysteine-HCl·H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. In a separate tube, add Na<sub>2</sub>S·9H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. Gas both solutions with 100% N<sub>2</sub> and cap tubes. Autoclave both solutions for 15 min at 15 psi pressure–121°C using fast exhaust. Cool to 50°C. Aseptically combine the two solutions under 100% N<sub>2</sub>.

#### **Wolfe's Mineral Solution:**

##### **Composition per liter**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriloacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water.

Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### **Wolfe's Vitamin Solution:**

##### **Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution and L-cysteine-sulfide reducing agent, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically add the sterile vitamin solution and then the sterile L-cysteine-sulfide reducing agent. Adjust the pH to 7.2. Distribute aseptically and anaerobically into sterile tubes.

**Use:** For the cultivation and maintenance of *Clostridium saccharolyticum*, *Clostridium thermoaceticum*, and *Clostridium thermohydrosulfuricum*.

### **Methanobacteria Medium with Xylose, Yeast Extract, and Tryptone**

#### **Composition per liter:**

Pancreatic digest of casein .....	10.0g
Xylose .....	5.0g
Yeast extract .....	3.0g
Mineral solution 2 .....	50.0mL
Sodium carbonate solution .....	50.0mL
Mineral solution 1 .....	25.0mL
L-Cysteine-sulfide reducing agent .....	20.0mL
Wolfe's mineral solution .....	10.0mL
Vitamin solution .....	10.0mL
Resazurin (0.025% solution) .....	4.0mL

pH 7.2 ± 0.2 at 25°C

#### **Mineral Solution 1:**

##### **Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
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**Preparation of Medium:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Mineral Solution 2:**

##### **Composition per liter:**

NaCl .....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.6g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Sodium Carbonate Solution:**

##### **Composition per 100.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	8.0g
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**Preparation of Sodium Carbonate Solution:** Add  $\text{Na}_2\text{CO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**L-Cysteine-Sulfide Reducing Agent:**

**Composition per 20.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.3g
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ .....	0.3g

**Preparation of L-Cysteine-Sulfide Reducing Agent:**

Add L-cysteine-HCl·H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. In a separate tube, add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to 10.0mL of distilled/deionized water. Mix thoroughly. Gas both solutions with 100%  $\text{N}_2$  and cap tubes. Autoclave both solutions for 15 min at 15 psi pressure–121°C using fast exhaust. Cool to 50°C. Aseptically combine the two solutions under 100%  $\text{N}_2$ .

**Wolfe's Mineral Solution:**

**Composition per liter:**

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	3.0g
Nitriloacetic acid .....	1.5g
$\text{NaCl}$ .....	1.0g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$ .....	0.5g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.1g
$\text{CaCl}_2$ .....	0.1g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .....	0.01g
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ .....	0.01g
$\text{H}_3\text{BO}_3$ .....	0.01g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution and L-cysteine-sulfide reducing agent, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Aseptically add the sterile vitamin solution and then the sterile L-cysteine-sulfide reducing agent. Adjust the pH to 7.2. Distribute aseptically and anaerobically into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermobacteroides acetothylus*.

**Methanobacteria Medium with Yeast Extract, Sodium Acetate, and Methanol**

**Composition per liter:**

Glucose .....	5.0g
Sodium acetate .....	4.1g

Yeast extract .....	2.0g
Mineral solution 2 .....	50.0mL
Sodium carbonate solution .....	50.0mL
Mineral solution 1 .....	25.0mL
L-cysteine-sulfide reducing agent .....	20.0mL
Wolfe's mineral solution .....	10.0mL
Vitamin solution .....	10.0mL
Methanol .....	4.0mL
Resazurin (0.025% solution) .....	4.0mL
pH 7.2 ± 0.2 at 25°C	

**Mineral Solution 1:**

**Composition per liter:**

$\text{K}_2\text{HPO}_4$ .....	6.0g
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**Preparation of Mineral Solution 1:** Add  $\text{K}_2\text{HPO}_4$  to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Mineral Solution 2:**

**Composition per liter:**

$\text{NaCl}$ .....	12.0g
$\text{KH}_2\text{PO}_4$ .....	6.0g
$(\text{NH}_4)_2\text{SO}_4$ .....	6.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	2.4g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	1.6g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Sodium Carbonate Solution:**

**Composition per 100.0mL:**

$\text{Na}_2\text{CO}_3$ .....	8.0g
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**Preparation of Sodium Carbonate Solution:** Add  $\text{Na}_2\text{CO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**L-Cysteine-Sulfide Reducing Agent:**

**Composition per 20.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	300.0mg
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ .....	300.0mg

**Preparation of L-Cysteine-Sulfide Reducing Agent:**

Add L-cysteine-HCl·H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. In a separate tube, add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to 10.0mL of distilled/deionized water. Mix thoroughly. Gas both solutions with 100%  $\text{N}_2$  and cap tubes. Autoclave both solutions for 15 min at 15 psi pressure–121°C using fast exhaust. Cool to 50°C. Aseptically combine the two solutions under 100%  $\text{N}_2$ .

**Wolfe's Mineral Solution:**

**Composition per liter:**

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	3.0g
Nitriloacetic acid .....	1.5g
$\text{NaCl}$ .....	1.0g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$ .....	0.5g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .....	0.1g
$\text{CaCl}_2$ .....	0.1g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.1g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .....	0.01g
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ .....	0.01g
$\text{H}_3\text{BO}_3$ .....	0.01g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, L-cysteine-sulfide reducing agent, and methanol, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize methanol. Aseptically add 4.0mL of sterile methanol to cooled, sterile basal medium. Aseptically add the sterile vitamin solution and then the sterile L-cysteine-sulfide reducing agent. Adjust the pH to 7.2. Distribute aseptically and anaerobically into sterile tubes.

**Use:** For the cultivation and maintenance of *Butyrivacterium methylophilum*.

***Methanobacterium alcaliphilum*  
Medium**

**Composition** per liter:

NaHCO <sub>3</sub> .....	10.0g
Yeast extract .....	2.0g
Peptone .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.02g
Resazurin .....	1.0mg
Salt solution .....	5.0mL

pH 8.4 ± 0.2 at 25°C

**Salt Solution:****Composition** per 100.0mL:

Sodium EDTA·2H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.03g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
AlCl <sub>3</sub> ·6H <sub>2</sub> O .....	8.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	4.0mg
NiSO <sub>4</sub> ·6H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	2.7mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
H <sub>3</sub> BO <sub>3</sub> .....	2.0mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub>, yeast extract, peptone, and L-cysteine-HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 990.0mL. Gently heat and bring to boiling under O<sub>2</sub>-free 100% N<sub>2</sub>. Continue boiling until resazurin becomes pale, indicating partial reduction. Add the yeast extract, peptone, and L-cysteine-HCl·H<sub>2</sub>O and continue boiling under O<sub>2</sub>-free 100% N<sub>2</sub>

until resazurin becomes colorless, indicating complete reduction. Cool to room temperature under O<sub>2</sub>-free 100% N<sub>2</sub>. Add NaHCO<sub>3</sub> to 10.0mL of distilled/deionized water. Mix thoroughly. Gas with O<sub>2</sub>-free 100% N<sub>2</sub> in a sealed tube. Add reduced NaHCO<sub>3</sub> solution to cooled reduced medium. Distribute anaerobically into tubes. Cap with butyl rubber stoppers and secure with closures. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation and maintenance of *Methanobacterium alcaliphilum*.

***Methanobacterium* Enrichment Medium**

**Composition** per liter:

CaCO <sub>3</sub> .....	100.0g
K <sub>2</sub> HPO <sub>4</sub> .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> CO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Ethanol .....	10.0mL
Yeast autolysate .....	5.0mL

pH 7.2 ± 0.2 at 25°C

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	0.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.1g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Filter sterilize ethanol. Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and ethanol, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile ethanol, Na<sub>2</sub>CO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and enrichment of *Methanobacterium* species.

***Methanobacterium espanolae* Medium**

**Composition** per 1020.0mL:

Sodium acetate·3H <sub>2</sub> O .....	2.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.45g
K <sub>2</sub> HPO <sub>4</sub> .....	0.29g
KH <sub>2</sub> PO <sub>4</sub> .....	0.18g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
NaCl .....	0.05g
L-Cysteine-HCl .....	0.25g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 5.5 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitritotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense the medium anaerobically with 80% N<sub>2</sub> and 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.5–6.0. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Check the pH of the medium after autoclaving.

**Use:** For the cultivation of *Methanobacterium espanolae*.

***Methanobacterium formicicum* Medium****Composition per liter:**

NaCH <sub>3</sub> CO <sub>2</sub>	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.48g
NaCl	0.45g
L-Cysteine-HCl·H <sub>2</sub> O	0.27g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.06g
Fe(NH <sub>4</sub> )(SO <sub>4</sub> ) <sub>2</sub>	0.06g
MgSO <sub>4</sub>	45.0mg
Na <sub>2</sub> MoO <sub>4</sub>	24.0mg
K <sub>2</sub> HPO <sub>4</sub>	21.0mg
KH <sub>2</sub> PO <sub>4</sub>	21.0mg
Resazurin	1.0mg
Na <sub>2</sub> SeO <sub>3</sub>	0.2mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution	1.0mL

pH 6.8 ± 0.2 at 25°C

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	25.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room tem-

perature while sparging with 100%N<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O to the 100.0mL of anaerobic water. Mix thoroughly. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Autoclave for 20 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8 with concentrated HCl. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool medium while sparging with 100% N<sub>2</sub>. Prior to inoculation, aseptically and anaerobically add 1.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution per liter of medium. Repeat the addition of 1.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution per liter of medium every 48 hr during growth.

**Use:** For the cultivation of *Methanobacterium formicicum*.

***Methanobacterium* Medium****Composition per 1010.0mL:**

NaHCO <sub>3</sub>	4.0g
Sodium formate	2.0g
Sodium acetate	1.0g
Yeast extract	1.0g
L-Cysteine-HCl	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4g
NaCl	0.4g
NH <sub>4</sub> Cl	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	2.0mg
Resazurin	1.0mg
Sludge fluid	50.0mL
Fatty acid mixture	20.0mL
Trace elements solution SL-10	1.0mL

pH 6.7 ± 0.2 at 25°C

**Sludge Fluid:****Composition per 100.0mL:**

**Preparation of Sludge Fluid:** To 100.0mL of sludge from an anaerobic digester, add 0.4g of yeast extract. Sparge with 100% N<sub>2</sub> for a few minutes. Incubate at 37°C for 24 hr. Centrifuge the sludge at 13,000 × g for 15 min. Decant the clear supernatant solution. Sparge with 100% N<sub>2</sub> for a few minutes. Store in screw-capped bottles at room temperature in the dark.

**Fatty Acid Mixture:****Composition per 20.0mL:**

α-Methylbutyric acid	0.5g
Isobutyric acid	0.5g
Isovaleric acid	0.5g
Valeric acid	0.5g

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Adjust pH to 7.5 with concentrated NaOH.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL



**Preparation of Trace Elements Solution SL-10:**

Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium anaerobically under 80%  $\text{H}_2$  + 20%  $\text{CO}_2$ . Add components to distilled/deionized water and bring volume to 1010.0mL. Mix thoroughly. Sparge with 80%  $\text{H}_2$  + 20%  $\text{CO}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methanobacterium* species.

***Methanobacterium II Medium***  
**(DSMZ Medium 825)**

**Composition per 1050mL:**

Yeast extract	1.0g
$\text{NH}_4\text{Cl}$	1.0g
$\text{NaCl}$	0.6g
Cysteine-HCl· $\text{H}_2\text{O}$	0.5g
Sodium acetate	0.5g
$\text{K}_2\text{HPO}_4$	0.3g
$\text{KH}_2\text{PO}_4$	0.3g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.2g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.1g
KCl	0.1g
Resazurin	0.5mg
$\text{NaHCO}_3$ solution	40.0mL
Trace elements solution	10.0mL
Vitamin solution	10.0mL
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.0g
Nitrilotriacetic acid	1.5g
$\text{NaCl}$	1.0g
$\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$	0.5g
$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	0.18g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.18g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.1g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.1g
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.025g
$\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	0.02g
$\text{H}_3\text{BO}_3$	0.01g
$\text{Na}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$	0.01g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.01g
$\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl	10.0mg
Thiamine-HCl· $2\text{H}_2\text{O}$	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin $\text{B}_{12}$	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80%  $\text{H}_2$  + 20%  $\text{CO}_2$ . Filter sterilize.

 **$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  Solution:****Composition per 10.0mL:**

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	0.3g
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**Preparation of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Sparge with  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

 **$\text{NaHCO}_3$  Solution:****Composition per 100.0mL:**

$\text{NaHCO}_3$	10.0g
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**Preparation of  $\text{NaHCO}_3$  Solution:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Preparation of Medium:** Prepare and dispense medium under 80%  $\text{H}_2$  + 20%  $\text{CO}_2$  gas mixture. Add components, except  $\text{NaHCO}_3$  solution and  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, to 1.0L distilled/deionized water. Mix thoroughly. Sparge with 80%  $\text{H}_2$  + 20%  $\text{CO}_2$ . Dispense 5.0mL aliquots into Hungate tubes under 80%  $\text{H}_2$  + 20%  $\text{CO}_2$  gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Prior to use inject, for each 5.0mL medium, 0.2mL sterile  $\text{NaHCO}_3$  solution, and 0.05mL sterile  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution. The pH should be 7.0. After inoculation, pressurize culture vessels to 2 bar 80%  $\text{H}_2$  + 20%  $\text{CO}_2$  overpressure.

**Use:** For the cultivation of *Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanosarcina mazei*=*Methanococcus mazei* (*Methanosarcina frisia*), *Methanobacterium bryantii*, and *Methanobacterium oryzae* (*Methanobacterium* sp.).

***Methanobacterium ruminantium***  
**Medium**

**Composition per liter:**

$\text{NaHCO}_3$	6.0g
$\text{NaCl}$	2.0g
L-Cysteine-HCl· $\text{H}_2\text{O}$	1.0g
$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	1.0g
$\text{KH}_2\text{PO}_4$	1.0g
$\text{NH}_4\text{Cl}$	1.0g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.1g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1g
Resazurin	1.0mg
Rumen fluid	300.0mL
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution	10.0mL

6.8 ± 0.2 at 25°C

 **$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  Solution:****Composition per 10.0mL:**

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	0.25g
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**Preparation of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  Solution:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 80%  $\text{H}_2$  + 20%  $\text{CO}_2$ . Add components, except rumen fluid and  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, to distilled/deionized water and bring volume to 690.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Autoclave for 15 min at 15 psi pressure–121°C. Cool to

25°C. Aseptically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 300.0mL of sterile rumen fluid. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanobacterium ruminantium*.

***Methanobacterium subterraneum* Medium  
(DSMZ Medium 814)**

**Composition per liter:**

Na-formate .....	3.2g
Na-acetate .....	1.0g
Tryptone .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
NaCl .....	0.45g
NH <sub>4</sub> Cl .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.03g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.003g
Resazurin .....	0.5mg
Trace elements solution .....	10.0mL
Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Seven vitamin solution .....	1.0mL

pH 8.3 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Cysteine Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.25g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Trace Elements Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Seven Vitamin Solution:**

**Composition per liter:**

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
p-Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, seven vitamin solution, cysteine solution, and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 969.0mL. Mix thoroughly. Flush medium with N<sub>2</sub> for 5 min. Adjust medium pH to 8.3. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 1.0mL seven vitamin mix, 10.0mL cysteine solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O, and 10.0mL NaHCO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 8.3. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Methanobacterium subterraneum*.

***Methanobacterium thermoautotrophicum*  
Marburg Medium**

**Composition per liter:**

L-Cysteine-HCl·H <sub>2</sub> O .....	12.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	12.5g
KH <sub>2</sub> PO <sub>4</sub> .....	6.8g
NH <sub>4</sub> Cl .....	2.1g
Na <sub>2</sub> CO <sub>3</sub> (1M solution) .....	32.0mL
Trace elements solution .....	10.0mL
Resazurin (0.2% solution) .....	0.3mL

**Trace Elements Solution:**

**Composition per liter:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0g
Nitrilotriacetic acid .....	3.0g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with NaOH. Add remaining components. Mix thoroughly. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl·H<sub>2</sub>O and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add L-cysteine-HCl·H<sub>2</sub>O and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Anaerobically distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Methanobacterium thermoautotrophicum*.

***Methanobacterium thermoautotrophicum* Medium  
(DSMZ Medium 131)**

**Composition per liter:**

Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.5g
NaCl .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.08g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0mg
Resazurin .....	1.0mg
Vitamin solution .....	10.0mL
Trace elements solution .....	10.0mL
L-Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Ca-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
<i>p</i> -Aminobenzoic acid .....	1.0mg
Vitamin B <sub>12</sub> .....	0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.2g
NaCl .....	1.0g
Na <sub>2</sub> -EDTA .....	0.64g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.55g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.17g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.13g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> .....	0.05g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.018g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.011g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Elements Solution:** Add Na<sub>2</sub>-EDTA to 500.0mL distilled/deionized water. Mix thoroughly. Add other components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**L-Cysteine Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	1.5g
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**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except vitamin solution, L-cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 10.0mL vitamin solution, 10.0mL of sterile L-cysteine solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks. Alternately the medium can be distributed to tubes under anaerobic conditions and autoclaved in tubes prior to addition of substrate solution, vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution. Appropriate amounts of these solutions can then be added to each tube by syringes to yield the desired concentrations.

**Use:** For the cultivation of *Ectothiorhodospira shaposhnikovii* and *Methanothermobacter thermautotrophicus*.

***Methanobacterium thermoautotrophicum*  
Medium**

**Composition per liter:**

Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.5g
NaCl .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.08g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0mg
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
L-Cysteine-HCl solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition per liter:**

NaCl .....	1.0g
Disodium EDTA .....	0.64g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.55g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.17g
CuSO <sub>4</sub> .....	0.15g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.13g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.018g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.011g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Elements Solution:** Add disodium EDTA to 500.0mL of distilled/deionized water. Mix thoroughly to dissolve. Add remaining components. Bring volume to 1.0L with distilled/deionized water.

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Nicotinic acid .....	5.0mg
Robiflavin .....	5.0mg
Thiamine-HCl .....	5.0mg

Biotin .....	2.0mg
Folic acid.....	2.0mg
p-Aminobenzoic acid.....	1.0mg
Cyanocobalamin .....	0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly.

#### L-Cysteine-HCl Solution:

**Composition per 10.0mL:**

L-Cysteine-HCl.....	1.5g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	1.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile L-cysteine-HCl solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile L-cysteine-HCl solution and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanobacterium thermoautotrophicus* and *Methanoculleus oldenburgensis*.

#### *Methanobacterium thermoautotrophicum* Medium, Taylor and Pirt

**Composition per liter:**

Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl.....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
Nitritotriacetic acid .....	0.03g
CoCl <sub>2</sub> .....	0.02g
CaCl <sub>2</sub> .....	0.01g
FeSO <sub>4</sub> .....	0.01g
MgSO <sub>4</sub> .....	0.01g
MnSO <sub>4</sub> .....	0.01g
ZnSO <sub>4</sub> .....	2.0mg
Resazurin .....	1.0mg
AlK(SO <sub>4</sub> ) <sub>2</sub> .....	0.2mg
CuSO <sub>4</sub> .....	0.2mg
H <sub>3</sub> BO <sub>3</sub> .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> .....	0.2mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL

pH 7.2 ± 0.2 at 25°C

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanobacterium thermoautotrophicum*.

#### *Methanobacterium thermoautotrophicum* MS Medium

**Composition per liter:**

NaHCO <sub>3</sub> .....	8.4g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> Cl.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.84g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.4g
Reazurin .....	0.001g
Trace minerals solution .....	1.0mL

pH 7.1 ± 0.2 at 25°C

#### Trace Minerals Solution:

**Composition per liter:**

Disodium EDTA·2H <sub>2</sub> O.....	0.5g
ZnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.21g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.15g
AlK(SO <sub>4</sub> ) <sub>2</sub> .....	0.14g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>4</sub> .....	0.01g

**Preparation of Trace Minerals Solution:** Add components, except MnSO<sub>4</sub>·H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1. Add MnSO<sub>4</sub>·H<sub>2</sub>O. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 7.1 with 6N HCl. Anaerobically distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

**Use:** For the cultivation of *Methanobacterium thermoautotrophicum*.

#### *Methanobacterium wolfei* Medium

**Composition per liter:**

Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
Sodium acetate·3H <sub>2</sub> O .....	1.0g

L-Cysteine-HCl.....	0.5g
NaCl.....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.26g
KH <sub>2</sub> PO <sub>4</sub> .....	0.26g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.26g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
NaWO <sub>4</sub> .....	2.6mg
FeSO <sub>4</sub> .....	2.0mg
Resazurin.....	1.0mg
Vitamin solution.....	10.0mL
Trace elements solution.....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Trace Elements Solution:****Composition per liter:**

Nitriloacetic acid.....	9.5g
MgCl <sub>2</sub> .....	4.06g
FeCl <sub>2</sub> .....	0.99g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.12g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.024g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.023g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Adjust pH to 7.0 with 10N NaOH.

**Vitamin Solution:****Composition per liter:**

Calcium DL-pantothenate.....	5.0g
Vitamin B <sub>12</sub> .....	0.1g
Pyridoxine-HCl.....	10.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Methanobacterium wolfei*.

***Methanobrevibacter curvatus* Medium  
(DSMZ Medium 734)**

**Composition per 1010mL:**

NaCl.....	1.0g
KCl.....	0.5g
Casamino acids.....	0.5g
Yeast extract.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Na <sub>2</sub> SO <sub>4</sub> .....	0.15g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
Resazurin.....	0.5mg
NaHCO <sub>3</sub> solution.....	40.0mL

Dithionite solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
Selenite-tungstate solution.....	1.0mL
Seven vitamin solution.....	1.0mL
pH 7.4 ± 0.2 at 25°C	

**NaHCO<sub>3</sub> Solution:****Composition per 40.0mL:**

NaHCO <sub>3</sub> .....	5.8g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Selenite-Tungstate Solution:****Composition per liter:**

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Dithionite Solution:****Composition per 10mL:**

Na-dithionite.....	2.0mg
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**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Seven Vitamin Solution:****Composition per liter:**

Pyridoxine hydrochloride.....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	200.0mg
Nicotinic acid.....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate.....	100.0mg
<i>p</i> -Aminobenzoic acid.....	80.0mg
D(+)-Biotin.....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, seven vitamin solution, selenite-tungstate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 947.0mL. Mix thoroughly. Adjust pH to 7.6. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pres-

sure–121°C. Aseptically and anaerobically add 40.0mL NaHCO<sub>3</sub> solution, 1.0mL selenite-tungstate solution, 1.0mL seven vitamin solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. Adjust pH to 7.6. Prior to inoculation add dithionite solution (0.1mL per 10mL medium) as reductant.

**Use:** For the cultivation of *Methanobrevibacter curvatus*.

### ***Methanocalculus halotolerans* Medium (DSMZ Medium 905)**

#### **Composition per 1010mL:**

NaCl .....	50.0g
NH <sub>4</sub> Cl .....	1.0g
Na-acetate .....	0.5g
Yeast extract .....	0.5g
Trypticase .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
KCl .....	0.17g
Resazurin .....	0.5mg
Magnesium chloride solution .....	30.0mL
NaHCO <sub>3</sub> solution .....	20.0mL
Trace elements solution .....	10.0mL
Calcium chloride solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
L-Cysteine solution .....	10.0mL

pH 7.2–7.6 at 25°C

#### **Magnesium Chloride Solution:**

##### **Composition per 30.0mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.2g
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#### **Preparation of Magnesium Chloride Solution:**

Add MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Calcium Chloride Solution:**

##### **Composition per 10.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.6g
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**Preparation of Calcium Chloride Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **L-Cysteine Solution:**

##### **Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
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**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

##### **Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **NaHCO<sub>3</sub> Solution:**

##### **Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for

15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

#### **Trace Elements Solution:**

##### **Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, magnesium chloride solution, calcium chloride solution, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add 10.0mL L-cysteine-HCl·H<sub>2</sub>O solution and 20.0mL NaHCO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 7.5. Distribute into anaerobic tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter, 30.0mL magnesium chloride solution, 10.0mL calcium chloride solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. After inoculation pressurize vessels with 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to 1 bar overpressure and add sulfide from a sterile, anaerobic stock solution. The final pH of the medium should be 7.2–7.6.

**Use:** For the cultivation of *Methanocalculus halotolerans*.

### ***Methanocalculus pumilus* Medium (DSMZ Medium 892)**

#### **Composition per 1080mL:**

NaCl .....	10.0g
Yeast extract .....	2.0g
Trypticase .....	2.0g
NH <sub>4</sub> Cl .....	0.9g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.36g
Resazurin .....	0.5mg
Na <sub>2</sub> CO <sub>3</sub> solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	15.0mL
Cysteine-HCl·H <sub>2</sub> O .....	15.0mL
Vitamin solution .....	10.0mL
Trace elements solution .....	10.0mL

#### **Cysteine Solution:**

##### **Composition per 15.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 15.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 50.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 15mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 15.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and cysteine solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 30 min. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically distribute 5.0mL aliquots into Hungate tubes under 100% N<sub>2</sub>. Aseptically and anaerobically add per 5.0mL medium 0.25mL Na<sub>2</sub>CO<sub>3</sub> solution, 0.075mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 0.075mL L-cysteine solution. Mix thoroughly. Replace N<sub>2</sub> atmosphere with atmosphere of 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Repeat atmosphere replacement several times with overpressurization. The initial pH of 9.0 will decrease over a 30 min

period to 7.3–7.5. After inoculation use atmosphere of 80% H<sub>2</sub> + 20% CO<sub>2</sub> to 1.5 bar overpressure.

**Use:** For the cultivation of *Methanocalculus pumilus*.

***Methanococcoides* Medium****Composition** per liter:

NaCl .....	18.0g
NaHCO <sub>3</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.45g
Trimethylamine-HCl .....	3.0g
Trypticase .....	2.0g
Yeast extract .....	2.0g
Sodium acetate .....	1.0g
L-Cysteine-HCl .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
KCl .....	0.335g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly.

**Preparation of Medium:** Prepare the medium anaerobically under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methanococcoides methylutens*.

***Methanococcus deltae* Medium**

**Composition per liter:**

NaCl .....	35.0g
NaHCO <sub>3</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0g
NH <sub>4</sub> Cl .....	2.7g
Sodium acetate .....	2.5g
L-Cysteine-HCl .....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.13g
Resazurin .....	1.0mg
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.3mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
L-Cysteine-HCl solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 6.9 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly.

**L-Cysteine-HCl Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl .....	0.3g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile L-cysteine-HCl solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile L-cysteine-HCl solution and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanococcus deltae*.

***Methanococcus jannaschii* Medium**

**Composition per liter:**

NaCl .....	30.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.40g
MgCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.7g
NaHCO <sub>3</sub> .....	1.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
KCl .....	0.33g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g
Resazurin .....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.5mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
L-Cysteine-HCl solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 6.0 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
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Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.0. Mix thoroughly.

#### L-Cysteine-HCl Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl.....	0.5g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile L-cysteine-HCl solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile L-cysteine-HCl solution and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanococcus* species.

### *Methanococcus* McC Medium

#### Composition per 1100.0mL:

NaHCO <sub>3</sub> .....	5.0g
Yeast extract.....	2.0g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.5g
General salts solution.....	500.0mL
NaCl solution.....	75.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	20.0mL
K <sub>2</sub> HPO <sub>4</sub> solution.....	10.0mL
Trace minerals solution.....	10.0mL
Sodium acetate solution.....	10.0mL
Iron stock solution.....	5.0mL
Resazurin solution.....	1.0mL

#### General Salts Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.9g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.5g
NH <sub>4</sub> Cl.....	1.0g
KCl.....	0.67g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.28g

**Preparation of General Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### NaCl Solution:

##### Composition per 100.0mL:

NaCl.....	29.3g
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**Preparation of NaCl Solution:** Add NaCl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

NaOH.....	1 pellet
Na <sub>2</sub> S·9H <sub>2</sub> O.....	2.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Dissolve 1 pellet of NaOH in the anaerobic water. Weigh out a little more than 2.5g of Na<sub>2</sub>S·9H<sub>2</sub>O. Briefly rinse the crystals in distilled/deionized water. Dry the crystals by blotting on paper towels or filter paper. Add 2.5g of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals to 100.0mL of anaerobic NaOH solution. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Pressurize to 60kPa with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

#### K<sub>2</sub>HPO<sub>4</sub> Solution:

##### Composition per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	1.4g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Trace Minerals Solution:

##### Composition per liter:

Nitritotriacetic acid.....	1.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1g
Mn <sub>4</sub> ·2H <sub>2</sub> O.....	0.1g
Zn <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·7H <sub>2</sub> O.....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g

**Preparation of Trace Minerals Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

#### Sodium Acetate Solution:

##### Composition per 100.0mL:

Sodium acetate·3H <sub>2</sub> O.....	13.6g
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**Preparation of Sodium Acetate Solution:** Add sodium acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Iron Stock Solution:

##### Composition per 100.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
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**Preparation of Iron Stock Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to 5.0mL of distilled H<sub>2</sub>O containing 2 drops of concentrated HCl. Mix thoroughly. When the Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O has dissolved, bring the volume to 100.0mL with distilled/deionized water.

#### Resazurin Solution:

##### Composition per 10.0mL:

Resazurin.....	10.0mg
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1080.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Anaerobically distribute 9.8mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.2mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Methanococcus* species.

#### **Methanococcus McN Medium**

**Composition per 1100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
General salts solution .....	500.0mL
NaCl solution .....	75.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	10.0mL
Trace minerals solution .....	10.0mL
Iron stock solution .....	5.0mL
Resazurin solution .....	1.0mL

#### **General Salts Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.9g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.5g
NH <sub>4</sub> Cl .....	1.0g
KCl .....	0.67g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.28g

**Preparation of General Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **NaCl Solution:**

**Composition per 100.0mL:**

NaCl .....	29.3g
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**Preparation of NaCl Solution:** Add NaCl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.5g
NaOH .....	1 pellet

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Dissolve 1 pellet of NaOH in the anaerobic water. Weigh out a little more than 2.5g of Na<sub>2</sub>S·9H<sub>2</sub>O. Briefly rinse the crystals in distilled/deionized water. Dry the crystals by blotting on paper towels or filter paper. Add 2.5g of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals to 100.0mL of anaerobic NaOH solution. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Pressurize to 60kPa with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

#### **K<sub>2</sub>HPO<sub>4</sub> Solution:**

**Composition per 100.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	1.4g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **Trace Minerals Solution:**

**Composition per liter:**

Nitritotriacetic acid .....	1.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1g
Mn <sub>4</sub> ·2H <sub>2</sub> O .....	0.1g
Zn <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g

**Preparation of Trace Minerals Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

#### **Iron Stock Solution:**

**Composition per 100.0mL:**

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
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**Preparation of Iron Stock Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to 5.0mL of distilled H<sub>2</sub>O containing 2 drops of concentrated HCl. Mix thoroughly. When the Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O has dissolved, bring the volume to 100.0mL with distilled/deionized water.

#### **Resazurin Solution:**

**Composition per 10.0mL:**

Resazurin .....	10.0mg
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1080.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Anaerobically distribute 9.8mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.2mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Methanococcus* species.

#### **Methanococcus McNail Medium**

**Composition per 1100.0mL:**

NaHCO <sub>3</sub> .....	5.0g
L-Leucine .....	1.0g
L-Isoleucine .....	0.5g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
General salts solution .....	500.0mL
NaCl solution .....	75.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	10.0mL
Trace minerals solution .....	10.0mL
Sodium acetate solution .....	10.0mL
Pantoyllactone solution .....	10.0mL
Iron stock solution .....	5.0mL
Resazurin solution .....	1.0mL

#### **General Salts Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.9g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.5g

NH <sub>4</sub> Cl.....	1.0g
KCl.....	0.67g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.28g

**Preparation of General Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### NaCl Solution:

**Composition per 100.0mL:**

NaCl.....	29.3g
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**Preparation of NaCl Solution:** Add NaCl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 100.0mL:**

NaOH.....	1 pellet
Na <sub>2</sub> S·9H <sub>2</sub> O.....	2.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Dissolve 1 pellet of NaOH in the anaerobic water. Weigh out a little more than 2.5g of Na<sub>2</sub>S·9H<sub>2</sub>O. Briefly rinse the crystals in distilled/deionized water. Dry the crystals by blotting on paper towels or filter paper. Add 2.5g of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals to 100.0mL of anaerobic NaOH solution. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Pressurize to 60kPa with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

#### K<sub>2</sub>HPO<sub>4</sub> Solution:

**Composition per 100.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	1.4g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Trace Minerals Solution:

**Composition per liter:**

Nitritotriacetic acid.....	1.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1g
Mn <sub>4</sub> ·2H <sub>2</sub> O.....	0.1g
Zn <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·7H <sub>2</sub> O.....	0.025g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g

**Preparation of Trace Minerals Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

#### Sodium Acetate Solution:

**Composition per 100.0mL:**

Sodium acetate·3H <sub>2</sub> O.....	13.6g
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**Preparation of Sodium Acetate Solution:** Add sodium acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Pantoyllactone Solution:

**Composition per 100.0mL:**

Pantoyllactone.....	0.013g
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**Preparation of Pantoyllactone Solution:** Add pantoyllactone to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Iron Stock Solution:

**Composition per 100.0mL:**

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
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**Preparation of Iron Stock Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to 5.0mL of distilled H<sub>2</sub>O containing 2 drops of concentrated HCl. Mix thoroughly. When the Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O has dissolved, bring the volume to 100.0mL with distilled/deionized water.

#### Resazurin Solution:

**Composition per 10.0mL:**

Resazurin.....	10.0mg
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1080.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Anaerobically distribute 9.8mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.2mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Methanococcus* species.

### *Methanococcus* Medium

**Composition per liter:**

NaCl.....	18.0g
Mg <sub>2</sub> SO <sub>4</sub> ·7H <sub>2</sub> O.....	3.45g
MgCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.75g
Pancreatic digest of casein.....	2.0g
Yeast extract.....	2.0g
Sodium acetate.....	1.0g
L-Cysteine·HCl.....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
NH <sub>4</sub> HCO <sub>3</sub> .....	0.5g
KCl.....	0.335g
NH <sub>4</sub> Cl.....	0.225g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14g
Calcium DL-pantothenate.....	5.0mg
Na <sub>2</sub> SeO <sub>3</sub> .....	2.0mg
FeSO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0mg
Resazurin.....	1.0mg
Trace minerals stock solution.....	10mL

pH 6.5 ± 0.2 at 25°C

#### Trace Minerals Stock Solution:

**Composition per liter:**

Nitritotriacetic acid.....	1.5g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
CoCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> .....	0.01g

#### Preparation of Trace Minerals Stock Solution:

Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Prepare medium anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>CO<sub>3</sub>, L-cysteine-HCl, and Na<sub>2</sub>S-9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Cool while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add Na<sub>2</sub>CO<sub>3</sub>, L-cysteine-HCl, and Na<sub>2</sub>S-9H<sub>2</sub>O. Dispense into tubes, bottles, or flasks under an atmosphere of 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Seal with butyl rubber stoppers secured with aluminum crimp seals. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Pressurize the head space to 69kPa with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Methanococcus* species.

#### *Methanococcus vannielii* Medium

##### **Composition** per 1020.0mL:

Solution A .....	500.0mL
Inorganic salts solution .....	500.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	10.0mL

##### **Solution A:**

##### **Composition** per 500.0mL:

Sodium formate .....	10.0g
Phenol Red .....	3.0mg
Methylene Blue .....	2.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

##### **Inorganic Salts Solution:**

##### **Composition** per 500.0mL:

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	1.45g
NH <sub>4</sub> Cl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Nitritotriacetic acid .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.6mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.9mg
ZnCl <sub>2</sub> .....	0.9mg
H <sub>3</sub> BO <sub>3</sub> .....	0.17mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.09mg

**Preparation of Inorganic Salts Solution:** Add nitritotriacetic acid to 250.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 500.0mL. Filter sterilize.

##### **Na<sub>2</sub>S-9H<sub>2</sub>O Solution:**

##### **Composition** per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

##### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

##### **Composition** per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	2.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically combine 500.0mL of sterile inorganic salts solution, 500.0mL of sterile solution A, 10.0mL of sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Methanococcus vannielii* from marine mud.

#### *Methanococcus vannielii* Medium

##### **Composition** per liter:

Sodium formate .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.48g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.38g
NH <sub>4</sub> Cl .....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	7.5mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	7.5mg
Na <sub>2</sub> SeO <sub>3</sub> .....	1.7mg
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL

##### **Na<sub>2</sub>S-9H<sub>2</sub>O Solution:**

##### **Composition** per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O .....	0.15g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S-9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL of sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanococcus vannielii*.

#### *Methanococcus voltae* BD Medium

##### **Composition** 1003.0mL:

L-Leucine .....	50.0g
NaCl .....	18.0g
Sodium panthothenate .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.48g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.75g
CH <sub>3</sub> COONa·3H <sub>2</sub> O .....	1.0g
KCl .....	0.34g
NH <sub>4</sub> Cl .....	0.26g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> PO <sub>4</sub> .....	0.14g
L-Isoleucine .....	0.1g
L-Cysteine/Na <sub>2</sub> S solution .....	17.5mL
Trace minerals solution .....	10.0mL
Vitamin solution .....	10.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	6.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	4.5mL

##### **Trace Minerals Solution:**

##### **Composition** per liter:

Nitritotriacetic acid .....	1.5g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Resazurin .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·5H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Minerals Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### **Vitamin Solution:**

##### **Composition per liter:**

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.5mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### **L-Cysteine/Na<sub>2</sub>S Solution:**

##### **Composition per liter:**

L-Cysteine-HCl .....	1.25g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.25g

**Preparation of L-Cysteine/Na<sub>2</sub>S Solution:** Add L-Cysteine-HCl and Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

##### **Composition per 100.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	1.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:**

##### **Composition per 100.0mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
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**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine/Na<sub>2</sub>S solution, Na<sub>2</sub>CO<sub>3</sub> solution, and FeSO<sub>4</sub>·7H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Gently heat and bring to boiling while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add 17.5mL of L-cysteine/Na<sub>2</sub>S·9H<sub>2</sub>O solution and 6.0mL of Na<sub>2</sub>CO<sub>3</sub> solution. Anaerobically dispense into tubes or bottles in 10.0mL aliquots under an atmosphere of 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Seal with butyl rubber stoppers secured with aluminum crimp seals. Autoclave for 15 min at 15 psi pressure–121°C. Immediately prior to inoculation, aseptically

and anaerobically add 0.05mL of sterile FeSO<sub>4</sub>·7H<sub>2</sub>O solution to each tube.

**Use:** For the cultivation of *Methanococcus voltae*.

### ***Methanocorpusculum* Medium (DSMZ Medium 279)**

#### **Composition per liter:**

Na-acetate .....	4.0g
NaHCO <sub>3</sub> .....	4.0g
Na-formate .....	2.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.002g
Resazurin .....	0.001g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
Sludge fluid .....	50.0mL
Fatty acid mixture .....	20.0mL
L-Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 6.7-7.0	

#### **Sludge Fluid:**

##### **Composition per 500.0mL:**

Yeast extract .....	2.0g
Sludge .....	500.0mL

**Preparation of Sludge Fluid:** Add yeast extract to sludge from an anaerobic digester. Gas with nitrogen gas for a few minutes. Incubate at 37°C for 24 hours. Centrifuge the sludge at 13,000 x g. Autoclave for 15 min at 15 psi pressure–121°C. The resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at room temperature in the dark.

#### **Fatty Acid Mixture:**

##### **Composition per 20.0mL:**

Valeric acid .....	0.5g
Isovaleric acid .....	0.5g
α-Methylbutyric acid .....	0.5g
Isobutyric acid .....	0.5g
Distilled water .....	20.0mL

**Preparation of Fatty Acid Mixture:** Add components to 20.0mL distilled/deionized water. Mix thoroughly.

#### **Trace Elements Solution SL-10:**

##### **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

##### **Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### L-Cysteine Solution:

**Composition per 10.0mL:**

L-Cysteine-HCl·H<sub>2</sub>O ..... 0.5g

**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except L-cysteine solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Adjust pH to 6.8. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL L-cysteine solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% H<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure. Alternately, the medium without L-Cysteine solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-10, can be distributed to tubes anaerobically prior to autoclaving. After autoclaving in tubes the appropriate volumes of the individual solutions can be injected through the stoppers so that the final concentrations of the medium are achieved.

**Use:** For the cultivation of *Methanococcoides* spp. and *Methanobolus bombayensis*.

#### *Methanoculleus olentangyi* Medium

**Composition per liter:**

NaHCO<sub>3</sub> ..... 5.0g  
NH<sub>4</sub>Cl ..... 2.7g  
Sodium acetate ..... 2.5g  
NaCl ..... 0.61g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.3g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.13g  
Resazurin ..... 1.0mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.3mg  
Trace elements solution ..... 10.0mL  
Vitamin solution ..... 10.0mL  
L-Cysteine-HCl solution ..... 10.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL

pH 6.9 ± 0.2 at 25°C

#### Trace Elements Solution:

**Composition per liter:**

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitrilotriacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### Vitamin Solution:

**Composition per liter:**

Pyridoxine-HCl ..... 10.0mg  
Calcium DL-pantothenate ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### L-Cysteine-HCl Solution:

**Composition per 10.0mL:**

L-Cysteine-HCl ..... 0.3g

**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile L-cysteine-HCl solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile L-cysteine-HCl solution and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanoculleus olentangyi*.

#### Methanogen Enrichment Medium, Barker

**Composition per liter:**

CaCO<sub>3</sub> ..... 20.0g  
NH<sub>4</sub>Cl ..... 1.0g  
K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O ..... 0.4g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
Methanol ..... 20.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except methanol and CaCO<sub>3</sub>, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add filter-sterilized methanol solution. Mix thoroughly. Add 1.0g of CaCO<sub>3</sub> to each of 50.0mL screw-capped bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Fill each bottle to capacity with enrichment medium.

**Use:** For the cultivation of methanogenic bacteria.

### **Methanogen Medium**

**Composition** per 106.0mL:

CaCO <sub>3</sub> .....	10.0g
Calcium acetate .....	2.0g
NH <sub>4</sub> Cl .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.04g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	3.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	3.0mL

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.1g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	0.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution and Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 3.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 3.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and enrichment of acetate-utilizing methanogenic bacteria.

### **Methanogen Medium, Zeikus**

**Composition** per 1010.0mL:

Inorganic salts solution .....	500.0mL
Vitamin solution .....	500.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

### **Inorganic Salts Solution:**

**Composition** per 500.0mL:

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	1.45g
NH <sub>4</sub> Cl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Nitritotriacetic acid .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.6mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.9mg
ZnCl <sub>2</sub> .....	0.9mg
H <sub>3</sub> BO <sub>2</sub> .....	0.17mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	.09mg

**Preparation of Inorganic Salts Solution:** Add nitritotriacetic acid to 250.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 500.0mL. Filter sterilize.

### **Vitamin Solution:**

**Composition** per 500.0mL:

Pyridoxine-HCl .....	1.0mg
p-Aminobenzoic acid .....	0.5mg
Ca-D-pantothenate .....	0.5mg
Nicotinic acid .....	0.5mg
Riboflavin .....	0.5mg
Thiamine-HCl .....	0.5mg
Thioctic acid .....	0.5mg
Biotin .....	0.2mg
Folic acid .....	0.2mg
Vitamin B <sub>12</sub> .....	0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Filter sterilize.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 95% N<sub>2</sub> + 5% CO<sub>2</sub>. Aseptically and anaerobically combine 500.0mL of sterile inorganic salts solution, 500.0mL of sterile vitamin solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of methanogenic bacteria.

### ***Methanogenium aggregans* Medium (DSMZ Medium 321)**

**Composition** per liter:

Na-formate .....	5.0g
Na <sub>2</sub> CO <sub>3</sub> .....	1.5g
Na-acetate .....	1.0g
Trypticase .....	1.0g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
Resazurin .....	1.0mg
Mineral solution .....	50.0mL
Cysteine-solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution .....	10.0mL
Sludge fluid .....	5.0mL

pH 6.8 ± 0.2 at 25°C

### **Mineral Solution:**

**Composition** per liter:

NaCl .....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.16g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g

CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Cysteine Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	0.2g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Sludge Fluid:

##### Composition per 500.0mL:

Yeast extract .....	2.0g
Sludge .....	500.0mL

**Preparation of Sludge Fluid:** Add yeast extract to sludge from an anaerobic digester. Gas with nitrogen gas for a few minutes. Incubate at 37°C for 24 hours. Centrifuge the sludge at 13,000 x g. Autoclave for 15 min at 15 psi pressure–121°C. The resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at room temperature in the dark.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except sludge fluid, cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 5.0mL sterile sludge fluid, 10.0mL of sterile cysteine solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanocorpusculum aggregans*=*Methanogenium aggregans*.

### Methanogenium Alcohol Medium

##### Composition per 1003.0mL:

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.4g
Sodium acetate·3H <sub>2</sub> O .....	0.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

NaHCO <sub>3</sub> solution .....	60.0mL
2-Propanol .....	5.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	3.0mL
Cyanocobalamin solution .....	1.0mL
Selenite-molybdate-tungstate solution .....	1.0mL
Thiamine solution .....	1.0mL
Trace elements solution .....	1.0mL
Vitamin solution .....	1.0mL

#### Trace Elements Solution:

##### Composition per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1400.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	145.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	120.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	50.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	3.0mg
HCl (25%, w/v) .....	8.0mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Selenite-Molybdate-Tungstate Solution:

##### Composition per liter:

NaOH .....	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	33.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Selenite-Molybdate-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

##### Composition per liter:

NaHCO <sub>3</sub> .....	84.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.5g
NaOH .....	1 pellet

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Dissolve 1 pellet of NaOH in the anaerobic water. Weigh out a little more than 2.5g of Na<sub>2</sub>S·9H<sub>2</sub>O. Briefly rinse the crystals in distilled/deionized water. Dry the crystals by blotting on paper towels or filter paper. Add 2.5g of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals to 100.0mL of anaerobic NaOH solution. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Pressurize to 60kPa with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

**Preparation of 2-Propanol:** Filter sterilize 10.0mL of 2-propanol. Sparge with 100% N<sub>2</sub>.

#### Vitamin Solution:

##### Composition per liter:

Sodium 2-mercaptoethanesulfonate .....	0.25g
Pyridoxine-HCl .....	0.15g
Calcium pantothenate .....	0.1g
Nicotinic acid .....	0.1g



<i>p</i> -Aminobenzoic acid.....	40.0mg
Biotin .....	10.0mg
Potassium phosphate buffer (25mM solution, pH 7.0) .....	1.0L

**Preparation of Vitamin Solution:** Combine components. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### Thiamine Solution:

**Composition** per liter:

Thiamine-HCl .....	0.1g
Sodium phosphate buffer (0.1M solution, pH 3.6) .....	1.0L

**Preparation of Thiamine Solution:** Combine components. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### Cyanocobalamin Solution:

**Composition** per liter:

Cyanocobalamin .....	50.0mg
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**Preparation of Cyanocobalamin Solution:** Add cyanocobalamin to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, 2-propanol, Na<sub>2</sub>S·9H<sub>2</sub>O solution, cyanocobalamin solution, selenite-molybdate-tungstate solution, thiamine solution, trace elements solution, and vitamin solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 60.0mL of sterile NaHCO<sub>3</sub> solution, 5.0mL of sterile 2-propanol, 3.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 1.0mL of sterile cyanocobalamin solution, 1.0mL of sterile selenite-molybdate-tungstate solution, 1.0mL of sterile thiamine solution, 1.0mL of sterile trace elements solution, and 1.0mL of sterile vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Methanogenium* species.

#### *Methanogenium bourgense* Medium (DSMZ Medium 322)

**Composition** per liter:

Na-formate .....	5.0g
Na <sub>2</sub> CO <sub>3</sub> .....	1.5g
Na-acetate .....	1.0g
Trypticase peptone .....	1.0g
Yeast extract .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg
Cysteine-solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 6.0–7.0 at 25°C

#### Cysteine Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except cysteine solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 10.0mL of sterile cysteine solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanoculleus bourgensis*=*Methanogenium bourgense*.

#### *Methanogenium* CV Medium

**Composition** per liter:

NaCl .....	18.0g
Isopropanol .....	7.5g
NaHCO <sub>3</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.45g
Trypticase™ .....	2.0g
Yeast extract .....	2.0g
Sodium acetate .....	1.0g
KCl .....	0.335g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
L-Cysteine-HCl .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 6.8–7.0 at 25°C

#### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 6.8–7.0. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile vitamin solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile vitamin solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanogenium organophilum*.

**Methanogenium Medium****Composition** per liter:

NaHCO <sub>3</sub>	4.0g
Sodium acetate	4.0g
Sodium formate	2.0g
Yeast extract	1.0g
L-Cysteine-HCl	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4g
NaCl	0.4g
NH <sub>4</sub> Cl	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O	2.0mg
Resazurin	1.0mg
Sludge fluid	50.0mL
Fatty acid mixture	20.0mL
Trace elements solution SL-10	1.0mL

pH 6.7 ± 0.2 at 25°C

**Sludge Fluid:****Composition** per 100.0mL:

Sludge	100.0mL
Yeast extract	0.4g

**Preparation of Sludge Fluid:** To 100.0mL of sludge from an anaerobic digester, add 0.4g of yeast extract. Sparge with 100% N<sub>2</sub> for a few minutes. Incubate at 37°C for 24 hr. Centrifuge the sludge at 13,000 × g for 15 min. Decant the clear supernatant solution. Sparge with 100% N<sub>2</sub> for a few minutes. Store in screw-capped bottles at room temperature in the dark.

**Fatty Acid Mixture:****Composition** per 20.0mL:

α-Methylbutyric acid	0.5g
Isobutyric acid	0.5g

Isovaleric acid	0.5g
Valeric acid	0.5g

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Adjust pH to 7.5 with concentrated NaOH.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium anaerobically under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methanobacterium* species, *Methanococcus* species, *Methanocorpusculum* species, *Methanoculleus* species, *Methanogenium* species, *Methanoplanus* species, and *Thermotoga* species.

**Methanogenium olentangyi Medium**  
(DSMZ Medium 287)**Composition** per liter:

NaHCO <sub>3</sub>	5.0g
NH <sub>4</sub> Cl	2.7g
Na-acetate	2.5g
NaCl	0.61g
K <sub>2</sub> HPO <sub>4</sub>	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.3g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.3g
CaCl <sub>2</sub> ·H <sub>2</sub> O	0.14g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.13g
Resazurin	1.0mg
Trace elements solution	10.0mL
Vitamin solution	10.0mL
Cysteine-HCl·H <sub>2</sub> O solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL

pH 6.9 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrotri-acetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0 with KOH.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Cysteine-HCl·H<sub>2</sub>O Solution:

##### Composition per 10mL:

Cysteine-HCl·H <sub>2</sub> O	0.3g
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**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except cysteine-HCl·H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and vitamin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 6.9. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL cysteine-HCl·H<sub>2</sub>O solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% H<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Methanoculleus olentangyi*=*Methanogenium olentangyi*.

#### *Methanogenium thermophilicum* Medium

##### Composition per liter:

NaCl	11.7g
Pancreatic digest of casein	6.0g
Sodium formate	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	4.0g
NaHCO <sub>3</sub>	4.0g
Yeast extract	2.0g
L-Cysteine-HCl	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.25g
K <sub>2</sub> HPO <sub>4</sub>	0.14g
KH <sub>2</sub> PO <sub>4</sub>	0.14g

CaCl<sub>2</sub>·2H<sub>2</sub>O 0.075g

Resazurin	1.0mg
Vitamin solution	10.0mL

**Preparation of Medium:** Add components, except L-cysteine-HCl and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bubble the solution with a stream of oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 minutes. Adjust the pH to 7.2 with 2M KOH. Add the L-cysteine-HCl and Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Tube the medium under oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> in either serum tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. After sterilization, the medium may form a precipitate. Allow it to cool and mix thoroughly to bring the precipitate back into solution.

**Use:** For the cultivation and maintenance of *Methanogenium thermophilicum*.

#### *Methanohalobium* Medium

##### Composition per liter:

NaCl	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	4.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.33g
KCl	0.33g
KH <sub>2</sub> PO <sub>4</sub>	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.33g
NH <sub>4</sub> Cl	0.33g
Resazurin	1.0mg
NaHCO <sub>3</sub> solution	100.0mL
Trimethylamine-HCl solution	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Yeast extract solution	10.0mL
Vitamin solution	10.0mL
Trace elements solution SL-10	10.0mL
Na <sub>2</sub> CO <sub>3</sub> solution	variable

pH 7.4 ± 0.2 at 25°C

#### NaHCO<sub>3</sub> Solution:

##### Composition per 10.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trimethylamine-HCl Solution:

##### Composition per 20.0mL:

Trimethylamine-HCl	0.2g
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#### Preparation of Trimethylamine-HCl Solution:

Add trimethylamine-HCl to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Yeast Extract Solution:

##### Composition per 5.0mL:

Yeast extract	50.0mg
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Nicotinic acid	5.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub>	0.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, trimethylamine-HCl solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, yeast extract solution, vitamin solution, trace elements solution SL-10, and Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 840.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 100.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile trimethylamine-HCl solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL of sterile yeast extract solution, 10.0mL of sterile vitamin solution, and 10.0mL of sterile trace elements solution SL-10. Mix thoroughly. Add a sufficient quantity of sterile Na<sub>2</sub>CO<sub>3</sub> solution to bring the pH to 7.4.

**Use:** For the cultivation and maintenance of *Methanohalobium evestigatus*.

***Methanohalophilus euhalobius*  
Medium**

**Composition** per 1010.0mL:

NaCl	60.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.4g
Yeast extract	2.0g
KCl	1.0g
Disodium EDTA	0.5g
NH <sub>4</sub> Cl	0.5g

KH <sub>2</sub> PO <sub>4</sub>	0.4g
Resazurin	0.5mg
Trimethylamine solution	50.0mL
NaHCO <sub>3</sub> solution	40.0mL
CaCl <sub>2</sub> solution	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	15.0mL
L-Cysteine-HCl solution	15.0mL
Wolfe's mineral solution	10.0mL
Wolfe's vitamin solution	5.0mL

pH 6.8–7.0 at 25°C

**Trimethylamine Solution:****Composition** per 50.0mL:

Trimethylamine-HCl	10.0g
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**Preparation of Trimethylamine Solution:** Add trimethylamine-HCl to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition** per 40.0mL:

NaHCO <sub>3</sub>	4.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**CaCl<sub>2</sub> Solution:****Composition** per 20.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O	2.0g
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**Preparation of CaCl<sub>2</sub> Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 20.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**L-Cysteine-HCl Solution:****Composition** per 20.0mL:

L-Cysteine-HCl·H <sub>2</sub> O	0.6g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Wolfe's Mineral Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrotriactic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### Wolfe's Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Calcium DL-pantothenate	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except trimethylamine solution, NaHCO<sub>3</sub> solution, CaCl<sub>2</sub> solution solution, L-Cysteine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 860.0mL. Mix thoroughly. Adjust pH to 6.8–7.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile trimethylamine solution, 40.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile CaCl<sub>2</sub> solution, 15.0mL of sterile L-cysteine-HCl solution, and 15.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Methanohalophilus euhalobius*.

#### *Methanohalophilus halophilus* Medium

##### Composition per 1011.0mL:

NaCl	70.0g
KCl	0.33g
KH <sub>2</sub> PO <sub>4</sub>	0.33g
NH <sub>4</sub> Cl	0.33g
Yeast extract	0.05g
K <sub>2</sub> SO <sub>4</sub>	0.01g
Resazurin	1.0mg
Methylamine-HCl solution	20.0mL
MgCl <sub>2</sub> /CaCl <sub>2</sub> solution	10.0mL
NaHCO <sub>3</sub> solution	10.0mL
Vitamin solution	10.0mL
L-Cysteine-HCl solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Trace elements solution SL-10	1.0mL

pH 7.2 ± 0.2 at 25°C

#### MgCl<sub>2</sub>/CaCl<sub>2</sub> Solution:

##### Composition per 10.0mL:

MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.33g

**Preparation of MgCl<sub>2</sub>/CaCl<sub>2</sub> Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 10.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix

thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Nicotinic acid	5.0mg
<i>p</i> -Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

#### Methylamine-HCl Solution:

##### Composition per 20.0mL:

Methylamine-HCl	5.0g
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**Preparation of Methylamine-HCl Solution:** Add methylamine-HCl to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### L-Cysteine-HCl Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl	0.2g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.2g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except MgCl<sub>2</sub>/CaCl<sub>2</sub> solution, NaHCO<sub>3</sub> solution, vitamin solution, methylamine-HCl solution, L-cysteine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 840.0mL. Mix thoroughly. Adjust pH to 6.9–7.0. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile MgCl<sub>2</sub>/CaCl<sub>2</sub> solution, 10.0mL of sterile NaHCO<sub>3</sub> solution,

10.0mL of sterile vitamin solution, 20.0mL of sterile methy-  
lamine-HCl solution, 10.0mL of sterile L-cysteine-HCl solu-  
tion, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix  
thoroughly.

**Use:** For the cultivation and maintenance of *Methanohalo-  
philus halophilus*.

### *Methanohalophilus mahii* Medium

**Composition** per 1010.0mL:

NaCl	87.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	6.0g
NaHCO <sub>3</sub>	4.0g
Trypticase	2.0g
Yeast extract	2.0g
KCl	1.5g
NH <sub>4</sub> Cl	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.4g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.4g
Coenzyme M (mercaptoethane sulfonic acid)	0.2g
Resazurin	1.0mg
Trace elements solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Methanol	4.0mL

pH 7.0 ± 0.2 at 25°C

### **Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Methanohalophilus mahii*.

### *Methanohalophilus oregonense* Medium

**Composition** per 1010.0mL:

NaCl	29.0g
Trimethylamine-HCl	2.0g

Trypticase	2.0g
Yeast extract	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	1.7g
KCl	1.5g
NH <sub>4</sub> Cl	1.0g
Resazurin	1.0g
Coenzyme M (mercaptoethane sulfonic acid)	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.4g
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Trace elements solution	10.0mL
Na <sub>2</sub> CO <sub>3</sub> solution	variable

pH 8.5 ± 0.2 at 25°C

### **Trace Elements Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub>	1.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 8.5 with a sufficient quantity of sterile Na<sub>2</sub>CO<sub>3</sub> solution (approximately 0.1mL per 10.0mL of medium).

**Use:** For the cultivation and maintenance of *Methanohalophilus oregonense*.

### *Methanohalophilus zhilinae* Medium

**Composition** per liter:

NaCl	40.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g

Trypticase.....	2.0g
Yeast extract.....	2.0g
KCl.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
L-Cysteine-HCl.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
Resazurin.....	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.1mg
Trimethylamine-HCl solution.....	20.0mL
NaHCO <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> CO <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution.....	5.0mL

pH 9.2 ± 0.2 at 25°C

**Trimethylamine-HCl Solution:****Composition** per 20.0mL:

Trimethylamine-HCl.....	2.0g
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**Preparation of Trimethylamine-HCl Solution:**

Add trimethylamine-HCl to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	0.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	2.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.25g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except trimethylamine-HCl solution, NaHCO<sub>3</sub> solution, Na<sub>2</sub>CO<sub>3</sub> solution, L-cysteine-HCl, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Allow to cool to room temperature while sparging with 100% N<sub>2</sub>. Add L-cysteine-HCl. Distribute medium into tubes and seal. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 20.0mL of sterile trimethylamine-HCl solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Check pH.

**Use:** For the cultivation and maintenance of *Methanohalophilus zhilinae*.

**Methanol Agar  
(LMG Medium 72)****Composition** per liter:

Agar.....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.62g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
NaCl.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	34.0mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	1.0mg
Methanol.....	10.0mL
Trace elements solution.....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per liter:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	10.0mg
H <sub>3</sub> BO <sub>3</sub> .....	10.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	7.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol, to 990.0mL distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically add 10.0mL sterile methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Methylobacterium* spp., *Methylobacillus glycogens*, and *Methylophilus methylotrophus*.

**Methanol Ammonium Salts Medium****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
NH <sub>4</sub> Cl.....	0.5g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.26g
CaCl <sub>2</sub> .....	0.2g
Ferrous EDTA.....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	2.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	500.0μg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	400.0μg
EDTA.....	250.0μg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	50.0μg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	20.0μg
H <sub>3</sub> BO <sub>3</sub> .....	15.0μg

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.0μg
Methanol .....	5.0mL
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add Na<sub>2</sub>HPO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. In a separate container, add remaining components, except methanol, to distilled/deionized water and bring volume to 895.0mL. Mix thoroughly. Autoclave both solutions for 15 min at 15 psi pressure–121°C. Cool to room temperature. Filter sterilize methanol. Aseptically add the sterile phosphate solution and the sterile methanol to the cooled, sterile basal medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the maintenance and cultivation of *Methylomonas methylotrophus*.

#### Methanol Medium (ATCC Medium 436)

**Composition per liter:**

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	7.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	8.0mg
Biotin .....	0.2μg
Thiamine-HCl .....	0.2μg
Methanol .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Filter sterilize methanol. Aseptically add the sterile methanol to the cooled, sterile basal medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Ancylobacter* species, *Methanomonas methylovora*, and *Methylobacterium* species.

#### Methanol Medium for *Achromobacter*

**Composition per liter:**

NH <sub>4</sub> Cl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
NaCl .....	0.5g
MgSO <sub>4</sub> .....	0.2g
Yeast extract .....	0.2g
FeSO <sub>4</sub> .....	2.0mg
MnCl <sub>2</sub> .....	2.0mg
Methanol .....	20.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Filter sterilize methanol. Aseptically add the sterile methanol to the cooled, sterile basal medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Achromobacter methanophilus*, *Methylobacterium rhodesianum*, *Pseudomonas insueta*, and *Pseudomonas polysaccharogenes*.

#### Methanol Medium with 1% Peptone

**Composition per liter:**

Agar .....	15.0g
Peptone .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	7.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	8.0mg
Biotin .....	0.2μg
Thiamine-HCl .....	0.2μg
Methanol .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Filter sterilize methanol. Aseptically add the sterile methanol to the cooled, sterile basal medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Methylobacterium* species.

#### Methanol Mineral Salts Medium

**Composition per liter:**

Agar .....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
NH <sub>4</sub> Cl .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	2.0g
Yeast extract .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Fe <sub>2</sub> SO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Methanol .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Filter sterilize methanol. Aseptically add the sterile methanol to the cooled, sterile basal medium. Mix thoroughly. Aseptically distribute into sterile Petri dishes or sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas viscogena*.

#### Methanol Salts Medium

**Composition per liter:**

Agar, noble .....	20.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.62g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.05g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	70.0μg
H <sub>3</sub> BO <sub>3</sub> .....	10.0μg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	10.0μg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	10.0μg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0μg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	5.0μg



FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.0mg
Methanol .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 1.0mL of filter-sterilized methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Hyphomicrobium* species, *Methylobacillus glycogenes*, *Methylobacterium extorquens*, *Methylobacterium fujisawaense*, *Methylobacterium mesophilicum*, *Methylobacterium organophilum*, *Methylobacterium radiotolerans*, *Methylobacterium rhodesianum*, *Methylobacterium rhodium*, *Methylobacterium* species, *Methylobacterium zamani*, *Methylomonas* species, *Methylophilus methylotrophus*, *Methylovorus glucosotrophus*, *Paracoccus* species, *Protaminobacter thiaminophaga*, and *Pseudomonas insueta*.

#### Methanol Urea Mineral Salts Medium

##### Composition per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	2.13g
KH <sub>2</sub> PO <sub>4</sub> .....	1.36g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	2.5mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.5mg
Urea solution .....	30.0mL
Methanol .....	5.0mL

##### Urea Solution:

##### Composition per 50.0mL:

Urea .....	10.0g
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**Preparation of Urea Solution:** Add urea to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Methanol:** Filter sterilize 5.0mL of methanol using a teflon filter.

**Preparation of Medium:** Add components, except urea solution and methanol, to distilled/deionized water and bring volume to 965.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 30.0mL of sterile urea solution and 5.0mL of sterile methanol. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Hyphomicrobium vulgare*.

#### Methanol-Utilizing Bacteria Medium B

##### Composition per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	30.0mg
Ferric citrate .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg
Thiamine-HCl .....	0.4mg
Methanol .....	10.0mL
pH 7.1 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of methanol-utilizing bacteria.

#### Methanol-Utilizing Bacteria Medium D

##### Composition per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
Yeast extract .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	30.0mg
Ferric citrate .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg
Methanol .....	10.0mL

pH 9.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically adjust pH to 9.0 with filter-sterilized 10% NaCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Paracoccus alcaliphilus*.

#### Methanol-Utilizing Bacteria Medium E

##### Composition per 1010.0mL:

NaCl .....	30.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	30.0mg
Ferric citrate .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg
Thiamine-HCl .....	0.4mg
Methanol .....	10.0mL
Vitamin B <sub>12</sub> .....	10.0µg

pH 9.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically adjust pH to 9.0 with filter-sterilized 10% NaCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methylophaga marina* and *Methylophaga thalassica*.

#### Methanobolus Medium

##### Composition per liter:

NaCl .....	18.0g
NaHCO <sub>3</sub> .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.45g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.75g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
KCl .....	0.335g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	2.0mg

Resazurin .....	1.0mg
Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Methanol .....	5.0mL

pH 6.5 ± 0.2 at 25°C

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriloacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except methanol and Wolfe's vitamin solution, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically add sterile Wolfe's vitamin solution and sterile methanol. Adjust pH to 6.5. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Methanobolus siciliae* and *Methanobolus tindarius*.

**Methanobolus 2 Medium****Composition per liter:**

NaCl .....	18.0g
NaHCO <sub>3</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.45g
Trypticase .....	2.0g
Yeast extract .....	2.0g
Sodium acetate .....	1.0g
L-Cysteine-HCl .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
KCl .....	0.335g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·7H <sub>2</sub> O .....	2.0mg

Resazurin .....	1.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
Methanol solution .....	5.0mL

pH 6.8–7.0 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Methanol Solution:****Composition per 5.0mL:**

Methanol .....	5.0mL
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**Preparation of Methanol Solution:** Sparge 5.0mL of methanol with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 6.9–7.0. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile vitamin solution and 10.0mL of sterile methanol to each liter of medium or, using a syringe, inject the appropriate amount of sterile vitamin solution and sterile methanol into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanobolus siciliae*, *Methanobolus vulcani*, and *Methanosarcina* species.

***Methanolobus taylori* Medium  
(DSMZ Medium 490a)****Composition per liter:**

NaCl	29.0g
Yeast extract	2.0g
Trypticase peptone	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	1.7g
KCl	1.5g
NH <sub>4</sub> Cl	1.0g
Resazurin	1.0g
Mercaptoethanesulfonic acid (coenzyme M)	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.4g
Trace elements solution	10.0mL
Trimethylamine-HCl	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
NaHCO <sub>3</sub> solution	10.0mL

pH 8.5 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitritotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Trimethylamine Solution:****Composition per 10.0mL:**

Trimethylamine-HCl	2.0g
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**Preparation of Trimethylamine Solution:** Add trimethylamine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub>	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Should be freshly prepared.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub> gas mixture. Add components, except trimethylamine solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to 970.0mL distilled/deionized water. Mix thoroughly. Sparge for 20 min with 100% N<sub>2</sub>.

Adjust pH to 8.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL sterile trimethylamine solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Adjust pH to 8.5 using sterile NaHCO<sub>3</sub> solution, approximately 10.0mL per liter medium. Aseptically and anaerobically distribute to sterile tubes or flasks under 100% N<sub>2</sub>.

**Use:** For the cultivation of *Methanolobus taylorii*.

***Methanomicrobium* Medium****Composition per liter:**

NaHCO <sub>3</sub>	2.0g
Yeast extract	1.0g
Pancreatic digest of casein	1.0g
NaCl	0.6g
L-Cysteine-HCl·H <sub>2</sub> O	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
K <sub>2</sub> HPO <sub>4</sub>	0.3g
KH <sub>2</sub> PO <sub>4</sub>	0.3g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.13g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	8.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O	2.0mg
Rumen fluid, clarified	300.0mL
Fatty acid mixture	20.0mL
Wolfe's mineral solution	10.0mL
Wolfe's vitamin solution	10.0mL
Resazurin (0.1% solution)	1.0mL

pH 6.5 ± 0.2 at 25°C

**Fatty Acid Mixture:****Composition per liter:**

Valeric acid	0.7mL
Isovaleric acid	0.7mL
α-Methylbutyric acid	0.5mL
Isobutyric acid	0.5mL

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitritotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.1g
CaCl <sub>2</sub>	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl	10.0mg
Thiamine-HCl	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
Calcium pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Thioctic acid	5.0mg

Biotin .....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin .....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Distribute into tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methanomicrobium mobile*.

### Methanomicrobium mobile Medium (DSMZ Medium 161)

**Composition per liter:**

NaHCO <sub>3</sub> .....	2.0g
Yeast extract.....	1.0g
Trypticase.....	1.0g
NaCl.....	0.6g
Cysteine-HCl·H <sub>2</sub> O.....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.13g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.008g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.002g
Resazurin.....	0.001g
Rumen fluid, clarified.....	300.0mL
Fatty acid mixture.....	20.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL

pH 6.5 ± 0.2 at 25°C

### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitritotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

### Vitamin Solution:

**Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg

Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

### Fatty Acid Mixture:

**Composition per 20.0mL:**

Valeric acid.....	0.5g
Isovaleric acid.....	0.5g
α-Methylbutyric acid.....	0.5g
Isobutyric acid.....	0.5g
Distilled water.....	20.0mL

**Preparation of Fatty Acid Mixture:** Add components to 20.0mL distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except vitamin solution, to 990.0mL distilled/deionized water. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 6.5 with concentrated NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 10.0mL sterile vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks. Alternately the medium can be distributed to tubes under anaerobic conditions and autoclaved in tubes prior to addition of vitamin solution. Appropriate amounts of the vitamin solution can then be added to each tube by syringes. After inoculation, pressurize culture vessels to 2 bar 80% H<sub>2</sub> + 20% CO<sub>2</sub> overpressure.

**Use:** For the cultivation of *Methanomicrobium mobile*.

### Methanomicrobium paynteri Medium

**Composition per liter:**

3-(N-morpholino) propane sulfonic acid (MOPS buffer).....	20.93g
NaCl.....	6.31g
NaHCO <sub>3</sub> .....	5.0g
Sodium acetate·3H <sub>2</sub> O.....	4.14g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.40g
MgCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.75g
NH <sub>4</sub> Cl.....	1.5g
KCl.....	0.34g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	2.0mg
Resazurin.....	1.0mg
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
L-Cysteine-HCl solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL

pH 7.0 ± 0.2 at 25°C

### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
NaCl.....	1.0g
Nitritotriacetic acid.....	1.5g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

#### L-Cysteine-HCl Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl .....	0.5g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except MOPS buffer, L-cysteine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Sparge with O<sub>2</sub>-free 80% H<sub>2</sub> + 20% CO<sub>2</sub>. In a separate flask, add MOPS buffer to distilled/deionized water and bring volume to 90.0mL. Adjust pH to 7.0 with 2M KOH. Add the 90.0mL of MOPS solution to the 890.0mL of medium and continue sparging with O<sub>2</sub>-free 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile L-cysteine-HCl solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile L-cysteine-HCl solution and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanomicrobium paynteri*.

#### *Methanomicrococcus Medium* (DSMZ Medium 120b)

##### Composition 1112.0mL:

NaCl .....	2.25g
Yeast extract .....	2.0g

Casitone .....	2.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.002g
Resazurin .....	0.001g
NaHCO <sub>3</sub> solution .....	40.0mL
Methanol solution .....	20.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution .....	15.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	15.0mL
Na-acetate solution .....	10.0mL
Vitamin solution .....	10.0mL
Coenzyme M solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Vitamin Solution:

##### Composition per liter:

Pyridoxamine-HCl .....	10.0mg
Pantothenic acid .....	5.0mg
Riboflavin .....	5.0mg
Alpha-lipoic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Nicotinic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	3.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15

min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### Methanol Solution:

##### Composition per 100.0mL:

Methanol .....50.0mL

**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na-Acetate Solution:

##### Composition per 100.0mL:

Na-acetate .....25.0g

**Preparation of Na-Acetate Solution:** Add Na-acetate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Coenzyme M Solution:

##### Composition per 10.0mL:

Coenzyme M .....0.1g

**Preparation of Coenzyme M Solution:** Add coenzyme M to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### L-Cysteine Solution:

##### Composition per 100.0mL:

L-Cysteine-HCl·H<sub>2</sub>O .....3.0g

**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, methanol solution, L-cysteine-HCl·H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na-acetate solution, coenzyme M solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL vitamin solution, 40.0mL NaHCO<sub>3</sub> solution, 20.0mL methanol solution, 15.0mL L-Cysteine-HCl·H<sub>2</sub>O solution, 15.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL Na-acetate solution, 1.0mL coenzyme M solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% H<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Methanomicrococcus blatticola*.

#### Methanomonas Autotrophic Medium

##### Composition per liter:

NaNO<sub>3</sub> .....2.0g  
Na<sub>2</sub>HPO<sub>4</sub> .....0.21g  
MgSO<sub>4</sub>·7H<sub>2</sub>O .....0.2g  
NaH<sub>2</sub>PO<sub>4</sub> .....0.09g  
KCl .....0.04g  
CaCl<sub>2</sub> .....0.015g  
FeSO<sub>4</sub>·7H<sub>2</sub>O .....1.0mg  
ZnSO<sub>4</sub>·7H<sub>2</sub>O .....0.3mg  
CuSO<sub>4</sub>·5H<sub>2</sub>O .....0.2mg  
H<sub>3</sub>BO<sub>3</sub> .....0.06mg

MnSO<sub>4</sub>·H<sub>2</sub>O .....0.03mg  
MoO<sub>3</sub> .....0.015mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the autotrophic cultivation of *Methanomonas* species.

#### Methanopyrus Medium

##### Composition per liter:

NaCl .....11.80g  
MgCl<sub>2</sub>·6H<sub>2</sub>O .....4.50g  
MgSO<sub>4</sub>·7H<sub>2</sub>O .....1.75g  
Na<sub>2</sub>SO<sub>4</sub> .....0.81g  
CaCl<sub>2</sub>·2H<sub>2</sub>O .....0.78g  
KCl .....0.30g  
KH<sub>2</sub>PO<sub>4</sub> .....0.09g  
K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O .....0.07g  
Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O .....2.0mg  
(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O .....2.0mg  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> .....2.0mg  
Resazurin .....0.2mg  
Marine trace elements .....10.0mL  
Trace elements solution .....10.0mL  
Vitamin solution .....10.0mL  
NaHCO<sub>3</sub> solution .....10.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution .....10.0mL

pH 6.5 ± 0.2 at 25°C

#### Marine Trace Elements:

##### Composition per liter:

NaBr .....4.0g  
SrCl<sub>2</sub>·6H<sub>2</sub>O .....1.8g  
H<sub>3</sub>BO<sub>3</sub> .....1.3g  
Sodium silicate .....100.0mg  
NaF .....60.0mg  
KNO<sub>3</sub> .....40.0mg  
KI .....1.25mg  
Na<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O .....0.25mg

**Preparation of Marine Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

##### Composition per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O .....3.0g  
Nitrilotriacetic acid .....1.5g  
NaCl .....1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O .....0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O .....0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O .....0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O .....0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O .....0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O .....0.025g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O .....0.02g  
CuSO<sub>4</sub>·5H<sub>2</sub>O .....0.01g  
H<sub>3</sub>BO<sub>3</sub> .....0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O .....0.01g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O .....0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....10.0mg

Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

#### NaHCO<sub>3</sub> Solution:

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	0.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile vitamin solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile vitamin solution, sterile NaHCO<sub>3</sub> solution, and sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution into individual tubes containing medium. Check that final pH of medium is 6.5.

**Use:** For the cultivation and maintenance of *Methanopyrus kandleri*.

#### *Methanosarcina acetivorans* Medium

**Composition per liter:**

NaCl.....	23.4g
Agar.....	10.0g
MgSO <sub>4</sub> .....	6.3g
Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
Trimethylamine-HCl.....	3.0g
Yeast extract.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
KCl.....	0.8g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.6g
L-Cysteine-HCl·H <sub>2</sub> O.....	0.25g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
Resazurin.....	1.0mg
Wolfe's mineral solution.....	10.0mL

pH 7.2 ± 0.2 at 25°C

#### Wolfe's Mineral Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitriloacetic acid.....	1.5g

NaCl.....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O, to glass-distilled water and bring volume to 990.0mL. Mix thoroughly. Methanol or methylamine-HCl may be substituted for the trimethylamine-HCl at a concentration of 50 mM. Heat gently and bring to boiling. Adjust pH to 7.2 with 6N HCl. Autoclave for 5 min at 10 psi pressure–115°C. Cool to 50°C under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. If a large precipitate is present, add a small amount of HCl and mix thoroughly. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Distribute into tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Cap with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. A precipitate will form but resolubilizes as the medium cools. Invert tubes as they are cooling to facilitate resolubilization. Allow tubes to cool in a slanted position.

**Use:** For the cultivation and maintenance of *Methanococcoides methylutens* and *Methanosarcina acetivorans*.

#### *Methanosarcina barkeri* Medium

**Composition per liter:**

NaHCO <sub>3</sub> .....	2.5g
NaCl.....	0.46g
Yeast extract.....	0.24g
KH <sub>2</sub> PO <sub>4</sub> .....	0.23g
K <sub>2</sub> HPO <sub>4</sub> .....	0.23g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.23g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.09g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.06g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.0mg
Methanol.....	10.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Resazurin (0.01% solution).....	1.0mL

**Preparation of Methanol:** Filter sterilize 10.0mL of methanol.

#### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·5H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
Na <sub>2</sub> S <sub>4</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.25g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·7H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g

H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### Vitamin Solution:

##### Composition per liter:

Calcium DL-pantothenate .....	5.0g
Vitamin B <sub>12</sub> .....	0.1g
Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### L-Cysteine-HCl·H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	2.5g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Add L-cysteine-HCl·H<sub>2</sub>O to the 100.0mL of anaerobic water. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Autoclave for 20 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

NaOH .....	1 pellet
Na <sub>2</sub> S·9H <sub>2</sub> O .....	2.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100%N<sub>2</sub>. Dissolve 1 pellet of NaOH in the anaerobic water. Weigh out a little more than 2.5g of Na<sub>2</sub>S·9H<sub>2</sub>O. Briefly rinse the crystals in distilled/deionized water. Dry the crystals by blotting on paper towels or filter paper. Weigh out 2.5g of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals. Add to the 100.0mL of anaerobic NaOH solution. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Pressurize to 60kPa with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except methanol, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute 9.7mL volumes into anaerobic tubes. Autoclave for 20 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.1mL of sterile methanol, 0.1mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution, and 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Methanosarcina barkeri*.

### Methanosarcina BCYT Medium (DSMZ Medium 318)

#### Composition per liter:

KHCO <sub>3</sub> .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
NaCl .....	0.6g
Yeast extract .....	0.5g
Trypticase .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.08g
Resazurin .....	1.0mg
Cysteine-solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
Methanol .....	5.0mL

pH 6.8 ± 0.2 at 25°C

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.



**Cysteine Solution:****Composition** per 10.0mL:L-Cysteine-HCl·H<sub>2</sub>O ..... 0.3g

**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except methanol, vitamin solution, cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 100% CO<sub>2</sub>. Aseptically and anaerobically add 5.0mL filter sterilized methanol, 10.0mL vitamin solution, 10.0mL cysteine solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanosarcina* spp.

***Methanosarcina* DPB Medium****Composition** per 1001.0mL:

Sodium acetate·3H<sub>2</sub>O ..... 4.1g  
 NH<sub>4</sub>Cl ..... 1.4g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 1.3g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.3g  
 MgSO<sub>4</sub> ..... 0.5g  
 NaCl ..... 0.5g  
 L-Cysteine-HCl·H<sub>2</sub>O ..... 0.27g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.06g  
 Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> ..... 0.01g  
 Antifoam C ..... 10.0mL  
 Trace elements solution ..... 10.0mL  
 Vitamin solution ..... 10.0mL  
 Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 1.0mL

pH 6.8 ± 0.2 at 25°C

**Source:** Antifoam C is available from Sigma Chemical Co.

**Trace Elements Solution:****Composition** per liter:

MgSO<sub>4</sub>·5H<sub>2</sub>O ..... 3.0g  
 Nitrilotriacetic acid ..... 1.5g  
 NaCl ..... 1.0g  
 MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
 Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.25g  
 CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
 CaCl<sub>2</sub>·7H<sub>2</sub>O ..... 0.1g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
 CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Vitamin Solution:****Composition** per liter:

Calcium DL-pantothenate ..... 5.0g  
 Vitamin B<sub>12</sub> ..... 0.1g  
 Pyridoxine-HCl ..... 10.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Nicotinic acid ..... 5.0mg

Riboflavin ..... 5.0mg  
 Thiamine-HCl ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 100.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 25.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Bring 100.0mL of distilled/deionized water to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O to the 100.0mL of anaerobic water. Mix thoroughly. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Autoclave for 20 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8 with concentrated HCl. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool medium while sparging with 100% N<sub>2</sub>. Prior to inoculation, aseptically and anaerobically add 1.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution per liter of medium. Repeat the addition of 1.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution per liter of medium every 48 hr during growth.

**Use:** For the cultivation of *Methanosarcina* species.

***Methanosarcina frisia* Medium****Composition** per liter:

NaCl ..... 18.0g  
 NaHCO<sub>3</sub> ..... 5.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 4.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.45g  
 Trypticase ..... 2.0g  
 Yeast extract ..... 2.0g  
 Sodium acetate ..... 1.0g  
 KCl ..... 0.335g  
 NH<sub>4</sub>Cl ..... 0.25g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.14g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 0.14g  
 L-Cysteine-HCl ..... 0.5g  
 Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g  
 Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·7H<sub>2</sub>O ..... 2.0mg  
 Resazurin ..... 1.0mg  
 Trace elements solution ..... 10.0mL  
 Vitamin solution ..... 10.0mL  
 Methanol solution ..... 2.0mL

pH 6.8–7.0 at 25°C

**Trace Elements Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
 Nitrilotriacetic acid ..... 1.5g  
 NaCl ..... 1.0g  
 MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
 CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
 CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

#### Methanol Solution:

##### Composition per 10.0mL:

Methanol .....	10.0mL
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**Preparation of Methanol Solution:** Sparge 10.0mL of methanol with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except methanol solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Anaerobically distribute into tubes or flasks fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Anaerobically add 10.0mL of sterile methanol solution to each liter of medium or, using a syringe, inject the appropriate amount of sterile methanol solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Methanosarcina mazei*.

#### *Methanosarcina mazei* Alpha Basal Medium

##### Composition per liter:

NaHCO <sub>3</sub> .....	4.4g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> S·6H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
Sodium acetate·3H <sub>2</sub> O .....	0.27g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.08g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.04g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.5mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0mg
Resazurin .....	1.0mg
H <sub>3</sub> BO <sub>4</sub> .....	0.1mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
ZnCl <sub>2</sub> .....	0.1mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Prepare and dispense medium under 70% N<sub>2</sub> + 30% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Sparge with 70% N<sub>2</sub> + 30% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Methanosarcina mazei*.

#### *Methanosarcina mazei* Medium (DSMZ Medium 120)

##### Composition 1112.0mL:

NaCl .....	2.25g
Yeast extract .....	2.0g
Casitone .....	2.0g
NaHCO <sub>3</sub> .....	0.85g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.002g
Resazurin .....	0.001g
Methanol solution .....	20.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	15.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	15.0mL
Na-acetate solution .....	10.0mL
Vitamin solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 6.5–6.8 at 25°C

#### Vitamin Solution:

##### Composition per liter:

Pyridoxamine·HCl .....	10.0mg
Pantothenic acid .....	5.0mg
Riboflavin .....	5.0mg
Alpha-lipoic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thiamine·HCl·2H <sub>2</sub> O .....	5.0mg
Nicotinic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 100.0mL:**Na<sub>2</sub>S·9H<sub>2</sub>O ..... 3.0g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Methanol Solution:****Composition per 100.0mL:**

Methanol ..... 50.0mL

**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na-Acetate Solution:****Composition per 100.0mL:**

Na-acetate ..... 25.0g

**Preparation of Na-Acetate Solution:** Add Na-acetate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**L-Cysteine Solution:****Composition per 100.0mL:**L-Cysteine·HCl·H<sub>2</sub>O ..... 3.0g

**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, methanol solution, L-cysteine·HCl·H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na-acetate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL vitamin solution, 10.0mL NaHCO<sub>3</sub> solution, 20.0mL methanol solution, 15.0mL L-cysteine·HCl·H<sub>2</sub>O solution, 15.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL Na-acetate solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% H<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Methanosarcina mazei*.

***Methanosarcina* Medium  
(BCYT)**

**Composition per liter:**

KHCO<sub>3</sub> ..... 2.0g  
 NH<sub>4</sub>Cl ..... 1.0g  
 NaCl ..... 0.6g  
 Pancreatic digest of casein ..... 0.5g  
 Yeast extract ..... 0.5g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.3g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.08g  
 Resazurin ..... 1.0mg  
 Trace elements solution ..... 10.0mL  
 Vitamin solution ..... 10.0mL  
 L-Cysteine·HCl·H<sub>2</sub>O solution ..... 10.0mL

Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL  
 Methanol ..... 5.0mL

pH 6.8 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

Nitritotriacetic acid ..... 12.8g  
 FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 1.35g  
 NaCl ..... 1.0g  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.12g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
 ZnCl<sub>2</sub> ..... 0.1g  
 Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.026g  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.025g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.024g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.024g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.01g

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl ..... 10.0mg  
 Calcium DL-pantothenate ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Thiamine·HCl ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**L-Cysteine·HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**L-Cysteine·HCl·H<sub>2</sub>O ..... 0.25g

**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except vitamin solution, L-cysteine·HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile vitamin solution, 10.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Methanosarcina mazei* and *Methanosarcina thermophila*.

### **Methanosarcina Medium**

#### **Composition per liter:**

NaCl .....	2.25g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
NaHCO <sub>3</sub> .....	0.85g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Methanol solution .....	10.0mL
Vitamin solution .....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 6.5–6.8 at 25°C

#### **Methanol Solution:**

##### **Composition per 10.0mL:**

Methanol .....	5.0mL
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**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 10.0mL. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Vitamin Solution:**

##### **Composition per liter:**

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **L-Cysteine·HCl·H<sub>2</sub>O Solution:**

##### **Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.3g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

##### **Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Elements Solution SL-10:**

##### **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg

ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Add components, except methanol solution, vitamin solution, L-cysteine·HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile methanol, 10.0mL of sterile vitamin solution, 10.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Methanosarcina* species.

### **Methanosarcina MP Medium**

#### **Composition per 1015.0mL:**

NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
L-Cysteine·HCl .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Resazurin .....	1.0mg
Mineral solution .....	50.0mL
Trace elements solution .....	10.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Methanol .....	5.0mL

pH 6.8 ± 0.2 at 25°C

#### **Mineral Solution:**

##### **Composition per liter:**

NaCl .....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.16g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Trace Elements Solution:**

##### **Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

Na<sub>2</sub>CO<sub>3</sub> ..... 1.0g

**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.25g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl, Na<sub>2</sub>CO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add L-cysteine-HCl. Mix thoroughly. Adjust pH to 6.8–7.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Methanosarcina* species.

### ***Methanosarcina* Nitrogen-Fixing Medium**

**Composition per 1005.0mL:**

NaH<sub>2</sub>PO<sub>4</sub> ..... 1.38g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.34g  
MgCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
Yeast extract ..... 0.1g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g  
Resazurin ..... 0.001g  
Trace elements solution ..... 10.0mL  
Methanol ..... 2.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O ..... 2.0mL  
NaHCO<sub>3</sub> ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

#### **Trace Elements Solution:**

**Composition per liter:**

Nitrilotriacetic acid ..... 4.5g  
FeCl<sub>2</sub>·7H<sub>2</sub>O ..... 0.4g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.19g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.17g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g  
ZnCl<sub>2</sub> ..... 0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02g  
Na<sub>2</sub>MoO<sub>4</sub>·6H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Adjust pH to 7.0 with 10N NaOH. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Methanol:** Sparge 2-propanol with 100% N<sub>2</sub>. Filter sterilize.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 2.0g  
NaOH ..... 0.1g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O and NaOH to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### **NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO<sub>3</sub> ..... 1.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except methanol, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 2.0mL of sterile methanol, 2.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 2.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 7.0 by adding sterile anaerobic 10N NaOH. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of nitrogen-fixing *Methanosarcina* species.

### ***Methanosarcina semesiae* Medium (DSMZ Medium 865)**

**Composition per 1214mL:**

Solution A ..... 950.0mL  
NaHCO<sub>3</sub> solution ..... 50.0mL  
Solution E ..... 30.0mL  
Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 15.0mL  
Solution D ..... 10.0mL  
Solution F ..... 10.0mL  
Solution G ..... 10.0mL  
Trace elements solution ..... 10.0mL  
Vitamin solution ..... 10.0mL  
Methanol solution ..... 5.0mL  
Solution B ..... 1.0mL  
Solution C ..... 1.0mL  
Seven vitamin solution ..... 1.0mL  
Dithionite solution ..... 1.0mL

pH 7.2 ± 0.2 at 25°C

#### **Solution A:**

**Composition per 950.0mL:**

KH<sub>2</sub>PO<sub>4</sub> ..... 1.4g  
NH<sub>4</sub>Cl ..... 0.5g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### **Solution B:**

**Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg

ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

##### Composition per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Solution D:

##### Composition per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Solution E:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 150.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Solution F:

##### Composition per 10.0mL:

Na <sub>2</sub> -maleate.....	1.6g
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**Preparation of Solution F:** Add Na<sub>2</sub>-maleate to distilled/deionized water and bring volume to 150.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Solution G:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.25g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Seven Vitamin Solution:

##### Composition per liter:

Pyridoxine hydrochloride.....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate.....	100.0mg
<i>p</i> -Aminobenzoic acid.....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

#### Methanol Solution:

##### Composition per 100.0mL:

Methanol.....	50.0mL
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**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 20mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Dithionite Solution****Composition** per 10mL:

Na-dithionite ..... 0.25g

**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Combine component solutions A-G. Do not add NaHCO<sub>3</sub> solution, Na<sub>2</sub>S-9H<sub>2</sub>O solution, trace elements solution, vitamin solution, methanol solution, seven vitamin solution, and dithionite solution. Mix thoroughly. Flush medium with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 5 min. Distribute into serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. For every 10.0mL medium aseptically and anaerobically add from sterile anaerobic solution: 0.5mL NaHCO<sub>3</sub> solution, 0.1mL trace elements solution, 0.1mL vitamin solution, 0.01mL seven vitamin solution, 0.01mL dithionite solution, 0.15mL Na<sub>2</sub>S-9H<sub>2</sub>O solution, and 0.05mL methanol solution. Final pH should be 7.0.

**Use:** For the cultivation of *Methanosarcina semesiae*.

***Methanosarcina Thermophilic Medium***  
**(DSMZ Medium 164)**

**Composition** 1051.0mL:

NaCl .....	2.25g
Yeast extract .....	2.0g
Casitone .....	2.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.002g
Resazurin .....	0.001g
Rumen fluid, clarified .....	50.0mL
Methanol solution .....	10.0mL
Vitamin solution .....	10.0mL
NaHCO <sub>3</sub> .....	10.0mL
L-Cysteine solution .....	10.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 6.5-6.8 at 25°C

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Methanol Solution:****Composition** per 100.0mL:

Methanol ..... 50.0mL

**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 100.0mL.

Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S-9H<sub>2</sub>O Solution:****Composition** per 100.0mL:Na<sub>2</sub>S-9H<sub>2</sub>O ..... 3.0g

**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**NaHCO<sub>3</sub> Solution:****Composition** per 100.0mL:NaHCO<sub>3</sub> ..... 8.5g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**L-Cysteine Solution:****Composition** per 10.0mL:L-Cysteine-HCl·H<sub>2</sub>O ..... 0.3g

**Preparation of L-Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, methanol solution, L-cysteine-HCl·H<sub>2</sub>O solution, Na<sub>2</sub>S-9H<sub>2</sub>O solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL vitamin solution, 10.0mL NaHCO<sub>3</sub> solution, 10.0mL methanol solution, 10.0mL L-cysteine-HCl·H<sub>2</sub>O solution, 10.0mL Na<sub>2</sub>S-9H<sub>2</sub>O solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% H<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure. Alternately, the medium without vitamin solution, NaHCO<sub>3</sub> solution, methanol solution, L-Cysteine-HCl·H<sub>2</sub>O solution, Na<sub>2</sub>S-9H<sub>2</sub>O solution, and trace elements solution SL-10, can be distributed to tubes anaerobically prior to autoclaving. After autoclaving in tubes the appropriate volumes of the individual solutions can be injected through the stoppers so that the final concentrations of the medium are achieved.

**Use:** For the cultivation of *Methanosarcina thermophila*.

### Methanospaera Medium I

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	2.8g
Trypticase.....	2.0g
Yeast extract.....	2.0g
NH <sub>4</sub> Cl.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
NaCl.....	0.6g
Sodium acetate.....	0.5g
Sodium formate.....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.15g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.076g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0mg
Na <sub>2</sub> SeO <sub>4</sub> .....	1.9mg
Resazurin.....	1.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.7mg
Rumen fluid, clarified.....	100.0mL
Trace elements solution.....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Methanol solution.....	5.0mL
Vitamin solution.....	1.0mL

pH 6.5 ± 0.2 at 25°C

### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

### L-Cysteine-HCl·H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O.....	0.875g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.375g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Methanol Solution:

**Composition** per 10.0mL:

Methanol.....	10.0mL
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**Preparation of Methanol Solution:** Sparge 10.0mL of methanol with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Vitamin Solution:

**Composition** per 10.0mL:

Calcium-D-pantothenate.....	20.0mg
Nicotinamide.....	20.0mg
Pyridoxine-HCl.....	20.0mg
Riboflavin.....	20.0mg
Thiamine-HCl.....	20.0mg
Biotin.....	10.0mg
p-Aminobenzoic acid.....	1.0mg
Folic acid.....	0.5mg
Vitamin B <sub>12</sub> .....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Add components, except methanol solution, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 5.0mL of sterile methanol solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Methanospaera stadtmanae*.

### Methanospaera Medium II

**Composition** per 1005.5mL:

NaHCO <sub>3</sub> .....	3.0g
Sodium acetate·3H <sub>2</sub> O.....	3.0g
Trypticase.....	1.0g
Yeast extract.....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·7H <sub>2</sub> O.....	2.0mg
Resazurin.....	1.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O(1mM).....	0.1mg
Mineral solution 1.....	40.0mL
Mineral solution 2.....	40.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Methanol.....	5.0mL

pH 7.0 ± 0.2 at 25°C

### Mineral Solution 1:

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
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**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Mineral Solution 2:

**Composition** per liter:

NaCl.....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.16g



**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### Vitamin Solution:

**Composition** per liter:

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### L-Cysteine·HCl·H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

L-Cysteine·HCl·H <sub>2</sub> O .....	0.3g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Methanol Solution:

**Composition** per 10.0mL:

Methanol .....	10.0mL
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**Preparation of Methanol Solution:** Sparge 10.0mL of methanol with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except L-cysteine·HCl·H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and methanol solution, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature

while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 5.0mL of sterile methanol solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Methanospaera cuniculi*.

#### *Methanospirillum hungatei* JMA Medium

**Composition** per liter:

Sodium acetate .....	2.5g
NH <sub>4</sub> Cl .....	1.9g
Mineral solution 1 .....	75.0mL
Mineral solution 2 .....	75.0mL
Wolfe's mineral solution .....	10.0mL
Vitamin solution .....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
FeSO <sub>4</sub> ·6H <sub>2</sub> O solution .....	6.0mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	4.8mL
Resazurin .....	1.0mL
NiCl <sub>2</sub> ·6H <sub>2</sub> O solution .....	0.1mL

#### FeSO<sub>4</sub>·6H<sub>2</sub>O Solution:

**Composition** per 100.0mL:

FeSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.2g
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**Preparation of FeSO<sub>4</sub>·6H<sub>2</sub>O Solution:** Add 0.2g of FeSO<sub>4</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### NiCl<sub>2</sub>·6H<sub>2</sub>O Solution:

**Composition** per liter:

NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.2g
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**Preparation of NiCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add 1.2g of NiCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Mineral Solution 1:

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	39.0g
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**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Mineral Solution 2:

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	24.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.79g
NaCl .....	0.59g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Wolfe's Mineral Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### Vitamin Solution:

**Composition** per liter:

Calcium DL-pantothenate .....	5.0g
Vitamin B <sub>12</sub> .....	0.1g
Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.4mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>CO<sub>3</sub> Solution:

**Composition** per liter:

Na <sub>2</sub> CO <sub>3</sub> .....	0.84g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### L-Cysteine-HCl·H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	0.22g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, L-cysteine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 975.2mL. Mix thoroughly. Gently heat and bring to boiling. Cool while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 4.8mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, 10.0mL of sterile L-cysteine-HCl solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Methanospirillum hungatei*.

#### Methanospirillum hungatei Medium

**Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	0.4g
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Sodium formate .....	0.2g
Pancreatic digest of casein .....	0.2g
Yeast extract .....	0.2g
NaCl .....	0.05g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.03g
K <sub>2</sub> HPO <sub>4</sub> .....	0.02g
KH <sub>2</sub> PO <sub>4</sub> .....	0.02g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.02g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	6.0mg
Resazurin .....	0.1mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	1.0mL
Trace metal solution .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.03g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Vitamin Solution:

**Composition** per 1000.0mL:

Pyridoxine-HCl .....	1.0mg
<i>p</i> -Aminobenzoic acid .....	0.5mg
Calcium-D-pantothenate .....	0.5mg
Nicotinic acid .....	0.5mg
Riboflavin .....	0.5mg
Thiamine-HCl .....	0.5mg
Thioctic acid .....	0.5mg
Biotin .....	0.2mg
Folic acid .....	0.2mg
Vitamin B <sub>12</sub> .....	0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Trace Metal Solution:

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	9.0g
K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
NH <sub>4</sub> Cl .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Metal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and distribute medium anaerobically under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methanospirillum hungatei*.

#### Methanospirillum hungatei SAM Medium

**Composition** per liter:

Na <sub>2</sub> CO <sub>3</sub> .....	2.63
Sodium acetate·3H <sub>2</sub> O .....	2.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.45g

K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.18g
MgSO <sub>4</sub> ·9H <sub>2</sub> O.....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.06g
NaCl.....	0.05g
Na <sub>2</sub> CO <sub>3</sub> solution.....	20.0mL
Trace minerals solution.....	10.0mL
Vitamin solution.....	10.0mL
L-Cysteine/Na <sub>2</sub> S solution.....	10.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution.....	5.0mL
Resazurin solution.....	0.2mL

pH 7.1 ± 0.2 at 25°C

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition per liter:**

Na <sub>2</sub> CO <sub>3</sub> .....	100.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Bring 1.0L of distilled/deionized water to boiling. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into serum bottles fitted with butyl rubber stoppers and aluminum seals. Do not grease stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Store at room temperature in an anaerobic chamber.

**Trace Minerals Solution:****Composition per liter:**

Nitrolotriacetic acid.....	1.5g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.24g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	0.165g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.15g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Minerals Solution:** Add nitrolotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

**Vitamin Solution:****Composition per liter:**

Vitamin B <sub>12</sub> .....	0.1g
Pyridoxine·HCl.....	10.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine·HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.4mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**L-Cysteine/Na<sub>2</sub>S Solution:****Composition per liter:**

L-Cysteine·HCl.....	1.25g
Na <sub>2</sub> S·7H <sub>2</sub> O.....	1.25g

**Preparation of L-Cysteine/Na<sub>2</sub>S Solution:** Gently heat and bring 75.0mL of distilled/deionized water to boiling. Add L-cysteine·HCl. Adjust pH to 10.0 with 3*N* NaOH. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with

100% N<sub>2</sub>. Bring volume to 100.0mL with distilled/deionized water. Gently heat and bring to boiling. Cool to 60°C while sparging with 100% N<sub>2</sub>. Anaerobically distribute into serum bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**FeSO<sub>4</sub>·6H<sub>2</sub>O Solution:****Composition per 100.0mL:**

FeSO <sub>4</sub> ·6H <sub>2</sub> O.....	0.2g
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**Preparation of FeSO<sub>4</sub>·6H<sub>2</sub>O Solution:** Add 0.2g of FeSO<sub>4</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Resazurin Solution:****Composition per 10.0mL:**

Resazurin.....	10.0mg
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>CO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub> solution and L-cysteine/Na<sub>2</sub>S solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to 60°C while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add Na<sub>2</sub>CO<sub>3</sub> and L-cysteine/Na<sub>2</sub>S solution. Mix thoroughly. Anaerobically distribute 10.0mL volumes into anaerobic tubes. Autoclave for 20 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.2mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution to each tube. Mix thoroughly. Adjust pH to 7.1.

**Use:** For the cultivation of *Methanospirillum hungatei*.

***Methanospirillum SK Medium*****Composition per liter:**

NaCl.....	18.0g
Isopropanol.....	7.5g
NaHCO <sub>3</sub> .....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.45g
Trypticase.....	2.0g
Yeast extract.....	2.0g
Sodium acetate.....	1.0g
L-Cysteine·HCl.....	0.5g
KCl.....	0.335g
NH <sub>4</sub> Cl.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·7H <sub>2</sub> O.....	2.0mg
Resazurin.....	1.0mg
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL

pH 7.0–7.4 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrolotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g

H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

#### Vitamin Solution:

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Methanospirillum hungatei*.

#### Methanothermus fervidus Medium

**Composition per liter:**

Na <sub>2</sub> SO <sub>4</sub> .....	3.4g
NaHCO <sub>3</sub> .....	2.0g
Trypticase .....	2.0g
Yeast extract .....	2.0g
L-Cysteine-HCl .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Ni(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .....	2.0mg
Resazurin .....	1.0mg
Mineral solution 1 .....	37.5mL
Mineral solution 2 .....	37.5mL
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 6.5 ± 0.2 at 25°C

#### Mineral Solution 1:

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
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**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Mineral Solution 2:

**Composition per liter:**

NaCl .....	12.0g
K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.6g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

#### Vitamin Solution:

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Adjust pH to 6.5 with 10N H<sub>2</sub>SO<sub>4</sub>. Add NaHCO<sub>3</sub> and sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Anaerobically distribute 20.0mL of medium into 100mL alkali-rich soda lime glass bottles. Pressurize to 2 bar with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methanothermus fervidus*.

#### Methanotherx Medium

**Composition per liter:**

Sodium acetate .....	6.8g
KHCO <sub>3</sub> .....	4.0g
NH <sub>4</sub> Cl .....	1.0g
NaCl .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g

MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.08g
Resazurin	1.0mg
Trace elements solution	10.0mL
Vitamin solution	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

Nitritotriacetic acid	12.8g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	1.35g
NaCl	1.0g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.10g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.10g
ZnCl <sub>2</sub>	0.10g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.026g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.025g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.024g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.024g
H <sub>3</sub> BO <sub>3</sub>	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**L-Cysteine-HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O	0.3g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except vitamin solution, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile vitamin solution, 10.0mL of sterile L-

cysteine-HCl·H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Check that final pH is 7.0. Use 20% inoculum.

**Use:** For the cultivation and maintenance of *Methanosaeta concilii*.

**Methylamine Salts Medium****Composition per liter:**

Agar	15.0g
Methylamine-HCl	6.75g
K <sub>2</sub> HPO <sub>4</sub>	2.12g
KH <sub>2</sub> PO <sub>4</sub>	1.0g
Solution A	5.0mL
Solution B	1.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition per 100.0mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution B:****Composition per 100.0mL:**

MnSO <sub>4</sub> ·7H <sub>2</sub> O	0.05g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.05g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Methylobacterium extorquens* and *Pseudomonas* species.

**Methylobacterium Agar****Composition per liter:**

Agar	12.0g
KNO <sub>3</sub>	1.0g
Na <sub>2</sub> HPO <sub>4</sub>	0.23g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
NaH <sub>2</sub> PO <sub>4</sub>	0.07g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	70.0µg
H <sub>3</sub> BO <sub>3</sub>	10.0µg
MnSO <sub>4</sub> ·5H <sub>2</sub> O	10.0µg
MoO <sub>3</sub>	10.0µg
CuSO <sub>4</sub> ·5H <sub>2</sub> O	5.0µg
Methanol, filter sterilized	5.0mL

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 995.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 5.0mL of filter-sterilized methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylobacterium organophilum*.

**Methylobacterium Medium**  
(DSMZ Medium 125)

**Composition** per liter:

Agar .....	12.0g
KNO <sub>3</sub> .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.23g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.07g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	70.0μg
MoO <sub>3</sub> .....	10.0μg
H <sub>3</sub> BO <sub>3</sub> .....	10.0μg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	10.0μg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	5.0μg
Methanol .....	15.0mL

pH 4.0-5.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Acidomonas methanolica* and *Methanomonas methylovora*.

**Methylobacterium Medium**

**Composition** per liter:

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.62g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	34.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	1.0mg
Trace elements solution .....	1.0mL
Methanol, filter sterilized .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition** per liter:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	70.0mg
H <sub>3</sub> BO <sub>3</sub> .....	10.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	10.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	7.0mg
CoCl <sub>2</sub> ·H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	5.0mg

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylobacterium* species.

**Methylobacterium thiocyanatum Medium**  
(DSMZ Medium 805)

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	7.9g
Glucose .....	4.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
KSCN .....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Iron sulfate solution .....	1.0mL

pH 7.1 ± 0.1 at 25°C

**Iron Sulfate Solution:**

**Composition** per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
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**Preparation of Iron Sulfate Solution:** Add 0.2g of FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 10 min at 10 psi pressure–115°C.

**Use:** For the cultivation of *Methylobacterium thiocyanatum*.

**Methylocapsa acidophila Medium**  
(DSMZ Medium 922)

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	100.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Trace elements solution .....	1.0mL

pH 4.5-5.8 at 25°C

**Trace Elements Solution:**

EDTA .....	5.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
CuCl <sub>2</sub> ·5H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. The final pH should be 4.5-5.8. The medium is fairly weakly buffered so the pH should be checked before and after autoclaving. Incubate under atmosphere of 10-30% methane.

**Use:** For the cultivation of *Methylocapsa acidiphila*.

**Methylocapsa acidophila Medium**  
(DSMZ Medium 922)

**Composition** per liter:

KNO <sub>3</sub> .....	100.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	100.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Trace elements .....	1.0mL

pH 4.5-5.8 at 25°C

**Trace Elements:**

EDTA .....	5.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
CuCl <sub>2</sub> ·5H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15

psi pressure–121°C. The final pH should be 4.5–5.8. The medium is fairly weakly buffered so the pH should be checked before and after autoclaving. Incubate under atmosphere of 10–30% methane.

**Use:** For the cultivation and enhanced growth of *Methylocapsa acidophila*.

### *Methylococcus Medium*

#### **Composition per liter:**

Agar .....	8.0g
NaNO <sub>3</sub> (20% solution) .....	10.0mL
L-F salts solution .....	10.0mL
Sodium-potassium phosphate buffer .....	6.5mL
pH 7.1 ± 0.2 at 25°C	

#### **Sodium-Potassium Phosphate Buffer:**

##### **Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	136.0g
NaOH .....	28.8g

**Preparation of Sodium-Potassium Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1.

#### **L-F Salts Solution:**

##### **Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O (10% solution) .....	200.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O (10% solution) .....	20.0mL
FeSO <sub>4</sub> (10% solution) .....	10.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (1% solution) .....	4.9mL
H <sub>3</sub> BO <sub>3</sub> (1% solution) .....	0.6mL
MnSO <sub>4</sub> ·H <sub>2</sub> O (1% solution) .....	0.27mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (1% solution) .....	0.2mL

**Preparation of L-F Salts Solution:** Filter sterilize FeSO<sub>4</sub> solution immediately prior to use. Add all components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Methylococcus* species.

### *Methylophaga Agar*

#### **Composition per 103.0mL:**

Agar solution .....	50.0mL
Mineral base, 2× .....	50.0mL
Solution T .....	2.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL
Methanol .....	0.3mL
pH 7.3 ± 0.2 at 25°C	

#### **Agar Solution:**

##### **Composition per 500.0mL:**

Agar .....	15.0g
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**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

#### **Mineral Base, 2×:**

##### **Composition per 500.0mL:**

NaCl .....	24.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
KCl .....	0.5g

Bis-Tris buffer (bis[2-Hydroxyethyl]imino-tris[hydroxymethyl]-methane) .....	0.5g
Wolfe's mineral solution .....	10.0mL

**Preparation of Mineral Base, 2×:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

#### **Wolfe's Mineral Solution:**

##### **Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriloacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### **Solution T:**

##### **Composition per 100.0mL:**

NH <sub>4</sub> Cl .....	10.0g
Bis-Tris buffer (bis[2-Hydroxyethyl]imino-tris[hydroxymethyl]-methane) .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.7g
Ferric ammonium citrate .....	0.3g

**Preparation of Solution T:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Vitamin B<sub>12</sub> Solution:**

##### **Composition per 10.0mL:**

Vitamin B <sub>12</sub> .....	1.0μg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically mix 50.0mL of the sterile agar solution with 50.0mL of the sterile mineral base, 2×. Aseptically combine the sterile solution T and sterile Vitamin B<sub>12</sub> solution with the sterile mineral base. Filter sterilize methanol and add to basal medium. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylophaga marina*.

### *Methylophaga alcalica Agar* (DSMZ Medium 976)

#### **Composition per liter:**

NaCl .....	30.0g
Agar .....	20.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
KNO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22g
Na <sub>2</sub> CO <sub>3</sub> solution .....	50.0mL
Methanol solution .....	50.0mL
Trace elements solution .....	1.0mL
pH 9.5 ± 0.2 at 25°C	

# **Methanol Solution:**

## **Composition per 50.0mL:**

Methanol .....10.0mL

**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

# **Na<sub>2</sub>CO<sub>3</sub> Solution:**

## **Composition per 50.0mL:**

Na<sub>2</sub>CO<sub>3</sub> ..... 5.0g

**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

# **Trace Elements Solution:**

## **Composition per liter:**

Ferric citrate .....30.0mg  
CaCl<sub>2</sub>·2H<sub>2</sub>O .....30.0mg  
MgCl<sub>2</sub>·4H<sub>2</sub>O .....5.0mg  
ZnSO<sub>4</sub>·7H<sub>2</sub>O .....5.0mg  
CuSO<sub>4</sub>·5H<sub>2</sub>O .....0.5mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol solution and Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C. Aseptically add 50.0mL warm sterile Na<sub>2</sub>CO<sub>3</sub> solution and 50.0mL warm sterile methanol solution. Mix thoroughly. Pour into Petri dishes or aseptically distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylophaga alcalica*.

## ***Methylophaga* Medium**

### **Composition per liter:**

NaCl ..... 25.0g  
Agar ..... 20.0g  
Peptone ..... 10.0g  
Beef extract ..... 7.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0g  
Methanol, filter sterilized .....10.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Methylophaga marina* and *Methylophaga thalassica*.

## ***Methylophaga sulfidovorans* Medium (DSMZ Medium 951)**

### **Composition per liter:**

NaCl ..... 15.0g  
Na<sub>2</sub>CO<sub>3</sub> ..... 2.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.5g  
CaCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.33g  
KCl ..... 0.2g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.02g  
DMS (Dimethylsulphide) ..... 62.0mg

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.0mg  
Trace elements solution SL10 ..... 1.0mL  
Vitamin solution ..... 1.0mL

pH 7.5 ± 0.3 at 25°C

## **Trace Elements Solution SL-10:**

### **Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

## **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

## **Vitamin Solution:**

### **Composition per liter:**

Pyridoxine-HCl .....500.0mg  
Nicotinic acid .....200.0mg  
Thiamine .....100.0mg  
*p*-Aminobenzoic acid .....100.0mg  
Pantothenate .....50.0mg  
Biotin .....20.0mg  
Riboflavine .....10.0mg  
Vitamin B<sub>12</sub> .....10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Methylophaga sulfidovorans*.

## ***Methylophaga thalassica* Agar (LMG Medium 73)**

### **Composition per liter:**

NaCl .....25.0g  
Agar .....20.0g  
Peptone .....10.0g  
Lab Lemco beef extract .....7.0g  
K<sub>2</sub>HPO<sub>4</sub> .....1.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> .....1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except methanol, to 990.0mL distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically add 10.0mL sterile methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Methylophaga thalassica*.

## ***Methylosarcina quisquillarum* *Methylosarcina fibrata* Medium (DSMZ Medium 921)**

### **Composition per 1012.1mL:**

Solution 1 ..... 100.0mL  
Phosphate buffer ..... 10.0mL  
Solution 3 ..... 1.0mL



Trace elements .....	1.0mL
Solution 2 .....	0.1mL
pH 7.0 ± 0.2 at 25°C	

**Solution 1 (10x NMS Salts):****Composition per liter:**

KNO <sub>3</sub> .....	10.0g
MgSO <sub>4</sub> ·6H <sub>2</sub> O .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0g

**Preparation of Solution 1 (10x NMS Salts):** Add components to 700.0mL distilled/deionized water. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly.

**Solution 2 Fe EDTA:****Composition per liter:**

Fe EDTA .....	3.8g
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**Preparation of Solution 2 Fe EDTA:** Add Fe EDTA to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Solution 3 Sodium Molybdate:****Composition per liter:**

Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.26g
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**Preparation of Solution 3 Sodium Molybdate:** Add Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements:****Composition per 100mL:**

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	100.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	40.0mg
EDTA disodium salt .....	25.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.5mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.0mg

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Phosphate Buffer:****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	71.6g
KH <sub>2</sub> PO <sub>4</sub> .....	26.0g

**Preparation of Phosphate Buffer:** Add components to 800.0mL distilled/deionized water. Mix thoroughly. Adjust pH to 6.8. Bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C.

**Preparation of Medium:** Add 100.0mL solution 1 to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add 1.0mL of solution 3, 1.0mL of the trace elements, and 0.1mL of solution 2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C. Aseptically add 10.0mL phosphate buffer. Mix thoroughly. Aseptically distribute to sterile tubes or bottles.

**Use:** For the cultivation of *Methylosarcina fibrata* and *Methylosarcina quisquillarum*.

**Methylotrophic Arthrobacter  
Hyphomicrobium Medium  
(DSMZ Medium 939)**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	7.9g
Dimethylsulfone .....	1.9g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g

NH <sub>4</sub> Cl .....	0.8g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Trace elements solution .....	10.0mL
pH 7.2–7.5 at 25°C	

**Trace Elements Solution:****Composition per liter:**

EDTA .....	50.0g
NaOH .....	9.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> (MoO <sub>4</sub> ) .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Elements Solution:** Add Na<sub>2</sub>EDTA to 400.0mL distilled/deionized water. Mix thoroughly. Add 9.0g NaOH. Mix thoroughly. Individually dissolve each of the other components in 40.0mL distilled/deionized water. Add each of the other dissolved components to the EDTA solution, and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Adjust the pH to 6.0 with 1M NaOH.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Hyphomicrobium sulfonivorans*.

**Methylpyridine Medium****Composition per 1002.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	0.61g
KH <sub>2</sub> PO <sub>4</sub> .....	0.39g
KCl .....	0.25g
Yeast extract .....	0.1g
Wolfe's mineral solution .....	10.0mL
2-Methylpyridine .....	1.0mL

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Add components, except 2-methylpyridine, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. In a fume hood, aseptically add 1.0mL of 2-methylpyridine. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Use

polyurethane foam closures to eliminate odors caused by volatilization of 2-methylpyridine.

**Use:** For the cultivation of *Arthrobacter* species.

### MG Medium

#### Composition per liter:

NaCl	100.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.45g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
Sodium acetate	1.0g
KCl	0.335g
NH <sub>4</sub> Cl	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.14g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.14g
Resazurin	1.0mg
NaHCO <sub>3</sub> solution	80.0mL
Trimethylamine-HCl solution	20.0mL
Na <sub>2</sub> CO <sub>3</sub> solution	10.0mL
Trace elements solution	10.0mL
Vitamin solution	10.0mL
L-Cysteine-HCl solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL

pH 6.9 ± 0.2 at 25°C

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub>	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trimethylamine-HCl Solution:

##### Composition per 20.0mL:

Trimethylamine-HCl	5.0g
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**Preparation of Trimethylamine-HCl Solution:** Add trimethylamine-HCl to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>CO<sub>3</sub> Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub>	0.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to approximately 500.0mL distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with

additional distilled/deionized water. Adjust pH to 7.0 with KOH.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### L-Cysteine-HCl Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl	0.5g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, trimethylamine-HCl solution, Na<sub>2</sub>CO<sub>3</sub> solution, vitamin solution, L-cysteine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 860.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 20 min. Then sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 10 min. Anaerobically distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 80.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile trimethylamine-HCl solution, 10.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, 10.0mL of sterile vitamin solution, 10.0mL of sterile L-cysteine-HCl solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Methanohalobium* species, *Methanohalophilus halophilus*, and *Methanohalophilus* species.

### MG Medium

#### Composition per liter:

NaCl	150.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.45g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
Sodium acetate	1.0g
KCl	0.335g
NH <sub>4</sub> Cl	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.14g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.14g
Resazurin	1.0mg
NaHCO <sub>3</sub> solution	80.0mL
Trimethylamine-HCl solution	20.0mL
Na <sub>2</sub> CO <sub>3</sub> solution	10.0mL
Trace elements solution	10.0mL
Vitamin solution	10.0mL

L-Cysteine-HCl solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
pH 6.9 ± 0.2 at 25°C	

**NaHCO<sub>3</sub> Solution:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trimethylamine-HCl Solution:****Composition** per 20.0mL:

Trimethylamine-HCl.....	5.0g
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**Preparation of Trimethylamine-HCl Solution:** Add trimethylamine-HCl to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 10.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	0.5g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitritotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**L-Cysteine-HCl Solution:****Composition** per 10.0mL:

L-Cysteine-HCl.....	0.5g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, trimethylamine-HCl solution, Na<sub>2</sub>CO<sub>3</sub> solution, vitamin solution, L-cysteine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 860.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 20 min. Then sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 10 min. Anaerobically distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 80.0mL of sterile NaHCO<sub>3</sub> solution, 20.0mL of sterile trimethylamine-HCl solution, 10.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, 10.0mL of sterile vitamin solution, 10.0mL of sterile L-cysteine-HCl solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Methanohalophilus* species.

**MH IH Agar****Composition** per liter:

Solution A.....	490.0mL
Solution B.....	490.0mL
Supplement solution.....	20.0mL
pH 6.9 ± 0.2 at 25°C	

**Solution A:****Composition** per 490.0mL:

Beef infusion.....	300.0g
Acid hydrolysate of casein.....	17.5g
Agar.....	17.0g
Starch.....	1.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution B:****Composition** per 490.0mL:

Hemoglobin.....	10.0g
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**Preparation of Solution B:** Add hemoglobin to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Supplement Solution:****Composition** per liter:

Glucose.....	100.0g
L-Cysteine-HCl.....	25.9g
L-Glutamine.....	10.0g
L-Cystine.....	1.1g
Adenine.....	1.0g
Nicotinamide adenine dinucleotide.....	0.25g
Vitamin B <sub>12</sub> .....	0.1g
Thiamine pyrophosphate.....	0.1g

Guanine-HCl .....	0.03g
Fe(NO <sub>3</sub> ) <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
<i>p</i> -Aminobenzoic acid .....	0.013g
Thiamine-HCl .....	3.0mg

**Source:** The supplement solution IsoVitaleX enrichment is available from BD Diagnostic Systems. This enrichment may be replaced by supplement VX from BD Diagnostic Systems.

**Preparation of Supplement Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine cooled, sterile solution A and cooled, sterile solution B. Mix thoroughly. Adjust pH to 6.9 with sterile 1N HCl or sterile 1N KOH. Aseptically add 20.0mL of sterile supplement solution. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and differentiation of *Legionella* species.

### MH Medium

**Composition per liter:**

NaCl .....	60.7g
Agar .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	15.0g
Yeast extract .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.4g
Proteose peptone No. 3 .....	5.0g
KCl .....	1.5g
Glucose .....	1.0g
CaCl <sub>2</sub> .....	0.27g
NaHCO <sub>3</sub> .....	0.45g
NaBr .....	0.19g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Deleya salina* and *Volcaniella eurihalina*.

### MH Medium

**Composition per liter:**

NaCl .....	60.7g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	15.0g
Yeast extract .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.4g
Proteose peptone No. 3 .....	5.0g
KCl .....	1.5g
Glucose .....	1.0g
CaCl <sub>2</sub> .....	0.27g
NaHCO <sub>3</sub> .....	0.045g
NaBr .....	0.019g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Halomonas eurihalina*.

### MH Medium, 2%

**Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g

NaCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.025g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Glucose solution .....	20.0mL
Methanol, filter sterilized .....	20.0mL

pH 7.0–7.5 at 25°C

### Glucose Solution:

**Composition per 20.0mL:**

Glucose .....	1.5g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and methanol, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile glucose solution and 20.0mL of filter-sterilized methanol. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Methylobacillus fructoseoxidans* and *Methylophilus glucoseroxidans*.

### MH Medium, 10% (LMG Medium 270)

**Composition per liter:**

NaCl .....	81.0g
Agar .....	15.0g
Yeast extract .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	7.0g
Proteose peptone no.3 .....	5.0g
KCl .....	2.0g
Glucose .....	1.0g
CaCl <sub>2</sub> .....	0.54g
NaBr .....	26.0mg
NaHCO <sub>3</sub> solution .....	10.0mL

pH 7.5 ± 0.2 at 25°C

### NaHCO<sub>3</sub> Solution:

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	0.06g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically add 10.0mL sterile NaHCO<sub>3</sub> solution. Pour into sterile Petri dishes or aseptically distribute into sterile tubes.

**Use:** For cultivation and maintenance of *Chromohalobacter israelensis* and *Chromohalobacter canadensis*.

### MH Medium, 10%

**Composition per liter:**

NaCl .....	81.0g
Yeast extract .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	7.0g
Proteose peptone No. 3 .....	5.0g
KCl .....	2.0g
Glucose .....	1.0g
CaCl <sub>2</sub> .....	0.36g
NaBr .....	0.026g
NaHCO <sub>3</sub> solution .....	10.0mL

pH 7.5 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**NaHCO<sub>3</sub> ..... 0.06g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Zygomonas mobilis*, *Salinicoccus hispanicus*, *Salinicoccus roseus*, and *Pseudomonas bejerinckii*.

**MH Medium, 15%  
(LMG Medium 258)**

**Composition per liter:**

NaCl ..... 121.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 14.4g  
MgCl<sub>2</sub> ..... 10.5g  
Yeast extract ..... 10.0g  
Proteose peptone no.3 ..... 5.0g  
KCl ..... 3.0g  
Glucose ..... 1.0g  
CaCl<sub>2</sub> ..... 0.54g  
NaBr ..... 39.0mg  
NaHCO<sub>3</sub> solution ..... 10.0mL

pH 7.5 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**NaHCO<sub>3</sub> ..... 0.09g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL sterile NaHCO<sub>3</sub> solution. Aseptically distribute into sterile tubes or flasks.

**Use:** For cultivation and maintenance of *Bacillus halophilus*.

***Micrococcus/Sarcina* Medium**

**Composition per liter:**

Agar ..... 16.0g  
Pancreatic digest of casein ..... 5.0g  
Sodium succinate·6H<sub>2</sub>O ..... 2.0g  
Starch ..... 2.0g  
Yeast autolysate ..... 1.0g  
Sodium citrate·2H<sub>2</sub>O ..... 0.5g  
Sodium acetate·3H<sub>2</sub>O ..... 0.3g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.2g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Micrococcus luteus* and *Sarcina* species.

***Microcycylus eburneus* Medium**

**Composition per liter:**

K<sub>2</sub>HPO<sub>4</sub> ..... 7.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 3.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 2.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
Yeast extract ..... 0.2g  
Thiamine-HCl ..... 0.2mg  
Biotin ..... 0.02mg  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.0µg  
MnSO<sub>4</sub>·4H<sub>2</sub>O ..... 2.0µg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Microcycylus eburneus*.

***Microcycylus major* Medium**

**Composition per liter:**

Glucose ..... 1.0g  
Peptone ..... 1.0g  
KNO<sub>3</sub> ..... 0.1g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.07g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.03g  
Trace elements solution ..... 1.0mL

**Trace Elements Solution:****Composition per liter:**

Disodium EDTA ..... 10.0g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 9.3g  
NaBO<sub>3</sub>·4H<sub>2</sub>O ..... 2.6g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.8g  
CaCl<sub>2</sub> ..... 1.2g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O ..... 1.0g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.08g  
Co(NO<sub>3</sub>)<sub>2</sub>·H<sub>2</sub>O ..... 0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Microcycylus major*.

***Microcycylus marinus* Medium**

**Composition per liter:**

NaCl ..... 23.5g  
MgCl<sub>2</sub> ..... 5.0g  
Na<sub>2</sub>SO<sub>4</sub> ..... 4.0g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.5g  
KCl ..... 0.7g  
NaHCO<sub>3</sub> ..... 0.2g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Microcycylus marinus*.

***Microcycylus* Medium**

**Composition per liter:**

Agar ..... 15.0g  
Glucose ..... 5.0g

Peptone.....	5.0g
Yeast extract.....	5.0g
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flectobacillus major* and *Microcycilus* species.

#### *Microcycilus/Spirosoma* Medium

<b>Composition per liter:</b>	
Agar .....	15.0g
Glucose .....	1.0g
Peptone.....	1.0g
Yeast extract.....	1.0g
pH 6.8–7.0 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Spirosoma linguale* and *Microcycilus* species.

#### *Microvirgula* Medium (DSMZ Medium 957)

<b>Composition per liter:</b>	
Na-succinate.....	1.5g
KNO <sub>3</sub> .....	1.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.82g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Yeast extract.....	0.25g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. The final pH should be 7.0.

**Use:** For the cultivation of *Microvirgula aerodenitrificans*.

#### MIL Medium (Motility Indole Lysine Medium)

<b>Composition per liter:</b>	
Peptone.....	10.0g
Pancreatic digest of casein.....	10.0g
L-Lysine-HCl.....	10.0g
Yeast extract.....	3.0g
Agar .....	2.0g
Dextrose .....	1.0g
Ferric ammonium citrate.....	0.5g
Bromcresol Purple .....	0.02g
pH 6.6 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder and prepared medium from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of members of the Enterobacteriaceae on the basis of motility, lysine decarboxylase activity, lysine deaminase activity, and indole production.

#### Milk Agar

**Composition per liter:**

Mixture A .....	500.0mL
Mixture B .....	500.0mL

**Mixture A:**

**Composition per 500.0mL:**

Instant nonfat milk .....	100.0g
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**Preparation of Mixture A:** Add instant nonfat milk to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool rapidly to 55°C.

**Mixture B:**

**Composition per 500.0mL:**

Agar .....	15.0g
Nutrient broth .....	12.5g
NaCl .....	2.5g

**Preparation of Mixture B:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool rapidly to 55°C.

**Preparation of Medium:** Aseptically combine cooled, sterile mixture A with cooled, sterile mixture B. Mix thoroughly. Pour into sterile Petri dishes in 20.0mL volumes.

**Use:** For the cultivation and estimation of the numbers of *Pseudomonas aeruginosa* in water by the membrane filter method.

#### Mineral Agar

**Composition per liter:**

NH <sub>4</sub> Cl .....	0.5g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	670.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	340.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	112.0mg
CaCl <sub>2</sub> .....	14.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	2.5mg
FeCl <sub>3</sub> .....	0.13mg
1,4-Dichlorobenzene .....	variable

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except 1,4-dichlorobenzene, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. After inoculation, place Petri dishes or tubes into a desiccator. Add a few crystals of 1,4-dichlorobenzene to the desiccator.

**Use:** For the cultivation of dichlorobenzene-degrading *Pseudomonas* species.

#### Mineral Base E for Autotrophic Growth

**Composition per liter:**

Noble agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.624g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
NaCl .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O solution .....	10.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O solution.....	10.0mL

Mineral solution .....	1.0mL
<i>p</i> -Aminobenzoic acid solution .....	1.0mL

**CaCl<sub>2</sub>·6H<sub>2</sub>O Solution:****Composition per liter:**

CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0g
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**Preparation of CaCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add 5.0g of CaCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
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**Preparation of MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 20.0g of MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

***p*-Aminobenzoic Acid Solution:****Composition per 10.0mL:**

<i>p</i> -Aminobenzoic acid .....	100.0mg
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**Preparation of *p*-Aminobenzoic Acid Solution:** Add *p*-aminobenzoic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Mineral Solution:****Composition per 1000.0mL:**

Disodium EDTA .....	1.58g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.7g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.16g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.052g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.047g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.047g

**Preparation of Medium:** Add components, except CaCl<sub>2</sub>·6H<sub>2</sub>O solution, MgSO<sub>4</sub>·7H<sub>2</sub>O solution, and *p*-aminobenzoic acid solution, to distilled/deionized water and bring volume to 979.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add in the following order: 10.0mL of sterile CaCl<sub>2</sub>·6H<sub>2</sub>O solution, 10.0mL of sterile MgSO<sub>4</sub>·7H<sub>2</sub>O solution, and 1.0mL of sterile *p*-aminobenzoic acid solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Incubate inoculated tubes in 50% CO<sub>2</sub>.

**Use:** For the autotrophic cultivation and maintenance of *Pseudomonas thermocarboxydovorans*.

### Mineral Base E for Heterotrophic Growth

**Composition per liter:**

Noble agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.624g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
NaCl .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O solution .....	10.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	10.0mL
Sodium pyruvate solution .....	10.0mL
Mineral solution .....	1.0mL
<i>p</i> -Aminobenzoic acid solution .....	1.0mL

**CaCl<sub>2</sub>·6H<sub>2</sub>O Solution:****Composition per liter:**

CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0g
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**Preparation of CaCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add 5.0g of CaCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
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**Preparation of MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 20.0g of MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Sodium Pyruvate Solution:****Composition per 10.0mL:**

Sodium pyruvate .....	2.0g
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**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

***p*-Aminobenzoic Acid Solution:****Composition per 10.0mL:**

<i>p</i> -Aminobenzoic acid .....	100.0mg
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**Preparation of *p*-Aminobenzoic Acid Solution:**

Add *p*-aminobenzoic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Mineral Solution:****Composition per 100.0mL:**

Disodium EDTA .....	1.58g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.7g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.16g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.052g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.047g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.047g

**Preparation of Medium:** Add components, except CaCl<sub>2</sub>·6H<sub>2</sub>O solution, MgSO<sub>4</sub>·7H<sub>2</sub>O solution, sodium pyruvate solution, and *p*-aminobenzoic acid solution to distilled/deionized water and bring volume to 969.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add in the following order: 10.0mL of the sterile CaCl<sub>2</sub>·6H<sub>2</sub>O solution, 10.0mL of the sterile MgSO<sub>4</sub>·7H<sub>2</sub>O solution, 10.0mL of sterile sodium pyruvate solution, and 1.0mL of sterile *p*-aminobenzoic acid solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the heterotrophic cultivation and maintenance of *Pseudomonas thermocarboxydovorans*.

**Mineral Lactate Medium****Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	1.13g
NaCl .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.88g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium lactate .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg
Trace elements solution .....	1.2mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

Disodium EDTA .....	50.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	22.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.54g

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.61g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	1.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with KOH.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Pseudomonas* species and *Spirillum* species.

#### Mineral Medium

##### Composition per liter:

Yeast extract .....	2.0g
Mineral base 5X .....	200.0mL
Trace elements solution SL-6 .....	1.0mL
Thiamine-HCl .....	3.0μg
Biotin .....	0.2μg

pH 6.8 ± 0.2 at 25°C

##### Mineral Base 5X:

##### Composition per liter:

NaCl .....	5.0g
NH <sub>4</sub> Cl .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.35g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.87g
CaCl <sub>2</sub> .....	0.05g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.25mg

**Preparation of Mineral Base 5X:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

##### Trace Elements Solution SL-6:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Arthrobacter* species.

#### Mineral Medium

##### Composition per liter:

NH <sub>4</sub> Cl .....	0.5g
Yeast extract .....	0.2g
1,4-Dichlorobenzene .....	0.1g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	670.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	340.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	112.0mg
CaCl <sub>2</sub> .....	14.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.5mg
FeCl <sub>3</sub> .....	0.13mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of dichlorobenzene-degrading *Pseudomonas* species.

#### Mineral Medium with 3-Aminophenol (DSMZ Medium 465f)

##### Composition per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.05g
Aminophenol solution .....	10.0mL
Trace elements solution SL-4 .....	1.0mL

pH 7.25 ± 0.2 at 25°C

##### Trace Elements Solution SL-4:

##### Composition per liter:

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

##### Trace Elements Solution SL-6:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

##### Aminophenol Solution:

##### Composition per 100.0mL:

3-Aminophenol .....	1.0g
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**Preparation of Aminophenol Solution:** Add 100.0mL boiling water to 1.0g aminophenol crystals. Stir the solution to mix thoroughly. Cool to room temperature. Sterilize by filtration.

**Preparation of Medium:** Add components, except aminophenol solution, to 990.0mL distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL aminophenol solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Arthrobacter* sp. and other aminophenol utilizing bacteria.

#### Mineral Medium with Antipyrin

##### Composition per liter:

Antipyrin .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	0.7g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	0.7g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
(NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g



(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.05g
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	0.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.2mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	0.2mg
KI.....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.04mg
Vitamin solution.....	20.0mL
pH 6.8–7.0 at 25°C	

**Vitamin Solution:****Composition** per 20.0mL:

Biotin.....	0.1mg
Vitamin B <sub>12</sub> .....	0.03mg

**Preparation of Vitamin Solution:** Add biotin and Vitamin B<sub>12</sub> to 20.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 6.8–7.0 with 1N NaOH. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add the sterile vitamin solution. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Phenylobacterium immobile*.

**Mineral Medium with Atrazine  
(DSMZ Medium 465i)**

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	3.5g
Na-citrate.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> .....	0.05g
Atrazine solution.....	10.0mL
Trace elements solution SL-4.....	1.0mL
pH 7.25 ± 0.2 at 25°C	

**Trace Elements Solution SL-4:****Composition** per liter:

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SL-6:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Atrazine Solution:****Composition** per 10.0mL:

Atrazine.....	100mg
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**Preparation of Atrazine Solution:** Add (2-chloro-4(ethylamino)-6-(isopropylamino)-1,3,5-triazine) to 10.0mL

methanol. Mix thoroughly. Shortly sonicate to reduce particle size.

**Preparation of Medium:** Add components, except atrazine solution, to 990.0mL distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL atrazine solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Pseudomonas* sp. and other atrazine-utilizing bacteria.

**Mineral Medium with Benzylcyanide  
(DSMZ Medium 465d)**

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O.....	0.05g
Benzylcyanide solution.....	10.0mL
Glucose solution.....	10.0mL
Trace elements solution SL-4.....	1.0mL
pH 7.25 ± 0.2 at 25°C	

**Trace Elements Solution SL-4:****Composition** per liter:

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

**Trace Elements Solution SL-6:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Glucose Solution:****Composition** per 10.0mL:

Glucose.....	1.8g
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**Preparation of Glucose Solution:** Add glucose to 10.0mL distilled/deionized water. Mix thoroughly. Filter sterilize.

**Benzylcyanide Solution:****Composition** per 100mL:

Benzylcyanide.....	0.12g
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**Preparation of Benzylcyanide Solution:** Add benzylcyanide to 10.0mL distilled/deionized water. Mix thoroughly. Do not sterilize.

**Preparation of Medium:** Add components, except benzylcyanide solution and glucose solution, to 980.0mL distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL glucose solution and 10.0mL benzylcyanide solution to the medium. Mix thoroughly. Aseptically distribute the medium to sterile tubes or flasks.

**Use:** For the cultivation of *Pseudomonas* sp., *Rhodococcus erythropolis*, and other benzylcyanide-utilizing bacteria.

**Mineral Medium, Brunner****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
Trace elements solution SL-4.....	10.0mL

pH 6.9 ± 0.2 at 25°C

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Alcaligenes* species, *Bacillus benzoevorans*, *Bacillus gordonae*, *Comamonas acidovorans*, *Hyphomicrobium* species, *Moraxella* species, *Nocardia* species, *Pseudomonas* species, *Rhodococcus* species, *Sphingomonas* species, and *Xanthobacter* species.

**Mineral Medium with Camphor****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	9.0g
Ferric ammonium citrate.....	5.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	3.0g
NH <sub>4</sub> Cl.....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Titriplex I.....	10.0mg
CoSO <sub>4</sub> .....	10.0μg
Camphor crumbs.....	variable

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except camphor crumbs, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Inoculate tubes or flasks and place in a dessicator jar in which crumbs of camphor will be evaporated.

**Use:** For the cultivation of bacteria that can utilize camphor as sole carbon source.

**Mineral Medium with Chloridazon****Composition per liter:**

Chloridazon.....	1.0g
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Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	0.7g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	0.7g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
(NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.05g
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	0.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.2mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	0.2mg
KI.....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.04mg
Vitamin solution.....	20.0mL

pH 6.8–7.0 at 25°C

**Vitamin Solution:****Composition per 20.0mL:**

Biotin.....	0.1mg
Vitamin B <sub>12</sub> .....	0.03mg

**Preparation of Vitamin Solution:** Add biotin and Vitamin B<sub>12</sub> to 20.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 6.8–7.0 with 1N NaOH. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add the sterile vitamin solution. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Phenylobacterium immobile*.

**Mineral Medium for Chemolithotrophic Growth****Composition per 985.0mL:**

Agar.....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.3g
NH <sub>4</sub> Cl.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
NaHCO <sub>3</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g
Ferric ammonium citrate solution.....	20.0mL
Trace elements solution SL-6.....	5.0mL

pH 6.8 ± 0.2 at 25°C

**Ferric Ammonium Citrate Solution:****Composition per 20.0mL:**

Ferric ammonium citrate.....	0.05g
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**Preparation of Ferric Ammonium Citrate Solution:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except ferric ammonium citrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile ferric ammonium citrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the chemolithotrophic growth and cultivation of a wide variety of bacteria.

**Mineral Medium  
with 2-Chlorobenzoate  
(DSMZ Medium 457a)**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Tween 80 .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements solution SL-4 .....	10.0mL
Chlorobenzoate solution .....	10.0mL

pH 7.4 ± 0.2 at 25°C

**Trace Elements Solution SL-4:**

**Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Trace Elements Solution SL-6:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Chlorobenzoate Solution:**

**Composition per liter:**

2-Chlorobenzoic acid .....	78.3g
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**Preparation of Chlorobenzoate Solution:** Add 2-chlorobenzoic acid to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Slowly add concentrated NaOH to adjust pH to 7.4. Filter sterilize.

**Preparation of Medium:** Add components, except chlorobenzoate solution, to 990.0mL distilled/deionized water. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL chlorobenzoate solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of chlorobenzoate-utilizing bacteria.

**Mineral Medium with Crude Oil**

**Composition per 100.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	0.45g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g

NaCl .....	0.01g
CaCl <sub>2</sub> .....	0.01g
FeCl <sub>3</sub> .....	0.002g
Crude oil .....	5.0mL

pH 7.2 ± 0.3 at 25°C

**Preparation of Medium:** Add components, except crude oil, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 5.0mL of filter-sterilized crude oil. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Acinetobacter baumannii*.

**Mineral Medium  
with Cyanuric Acid as Nitrogen Source  
(DSMZ Medium 465g)**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.05g
Cyanuric acid solution .....	10.0mL
Vitamin solution .....	10.0mL
Glycerol solution .....	10.0mL
Trace elements solution SL-4 .....	1.0mL

pH 7.25 ± 0.2 at 25°C

**Trace Elements Solution SL-4:**

**Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Trace Elements Solution SL-6:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin Solution:**

**Composition per liter:**

Vitamin B <sub>12</sub> .....	50.0mg
Pantothenic acid .....	50.0mg
Riboflavin .....	50.0mg
Alpha-lipoic acid .....	50.0mg
p-Aminobenzoic acid .....	50.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	50.0mg
Nicotinic acid .....	25.0mg
Nicotine amide .....	25.0mg
Biotin .....	20.0mg
Folic acid .....	20.0mg
Pyridoxamine-HCl .....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Cyanuric Acid Solution:****Composition** per 10.0mL:

Cyanuric acid ..... 645mg

**Preparation of Cyanuric Acid Solution:** Add cyanuric acid to 10.0mL distilled/deionized water. Mix thoroughly. Adjust pH to 7.0. Filter sterilize.

**Glycerol Solution:****Composition** per 10.0mL:

Glycerol ..... 5.5g

**Preparation of Glycerol Solution:** Add glycerol acid to distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cyanuric acid solution, glycerol solution, and vitamin solution to 1.0L distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL cyanuric acid solution, 10.0mL glycerol solution, and 10.0mL vitamin solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Gordonia rubripertincta*=*Rhodococcus rubropertinctus* and other cyanuric acid-utilizing bacteria.

**Mineral Medium with Dichlorobenzoate****Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub> ..... 2.78g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 2.78g  
 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0g  
 Hutner's mineral base ..... 20.0mL  
 2,4-Dichlorobenzoate solution ..... 10.0mL  
 pH 6.8 ± 0.2 at 25°C

**Hutner's Mineral Base:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 29.7g  
 Nitrilotriacetic acid ..... 10.0g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 3.34g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 99.0mg  
 (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> ..... 9.25mg  
 "Metals 44" ..... 50.0mL

**Preparation of Hutner's Mineral Base:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L.

**"Metals 44":****Composition** per 100.0mL:

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.1g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 EDTA ..... 0.25g  
 MnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.154g  
 CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.04g  
 Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
 Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O ..... 0.018g

**Preparation of "Metals 44":** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**2,4-Dichlorobenzoate Solution:****Composition** per 10.0mL:

2,4-Dichlorobenzoate ..... 5.0mg

**Preparation of 2,4-Dichlorobenzoate Solution:** Add 2,4-dichlorobenzoate to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except 2,4-dichlorobenzoate solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 6.8 with 1N KOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add the sterile 2,4-dichlorobenzoate solution. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Actinomyces viscosus*.

**Mineral Medium with Dichloromethane (DSMZ Medium 465c)****Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O ..... 3.5g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.5g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
 Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O ..... 0.05g  
 Bromothymol Blue ..... 50.0mg  
 Methanol ..... 10.0mL  
 Trace elements solution SL-4 ..... 1.0mL  
 Dichloromethane ..... variable  
 pH 7.25 ± 0.2 at 25°C

**Trace Elements Solution SL-4:****Composition** per liter:

EDTA ..... 0.5g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
 Trace elements solution SL-6 ..... 100.0mL

**Trace Elements Solution SL-6:****Composition** per liter:

H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.03g  
 Na<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O ..... 0.03g  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02g  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol and dichloromethane, to 1.0L distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Inoculate the medium and supply methanol via incubation atmosphere (up to 10.0mL methanol per liter medium). Subsequently feed small amounts of dichloromethane (toxic to bacteria at 2 mmol per liter or less) via incubation atmosphere. Readjust pH of the culture with sterile 1M NaOH as necessary.

**Use:** For the cultivation of *Methylophilus leisingeri*, *Rhizobium radiobacter*, and other dichloromethane-utilizing bacteria.

**Mineral Medium with 2,4-Dichlorophenoxyacetic Acid****Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O ..... 9.0g  
 Ferric ammonium citrate ..... 5.0g  
 MnSO<sub>4</sub>·H<sub>2</sub>O ..... 3.0g  
 NH<sub>4</sub>Cl ..... 2.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.5g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Titriplex I .....	10.0mg
CoSO <sub>4</sub> .....	10.0μg
2,4-Dichlorophenoxyacetic acid solution.....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**2,4-Dichlorophenoxyacetic Acid Solution:****Composition** per 10.0mL:

2,4-Dichlorophenoxyacetic acid .....	0.5g
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**Preparation of 2,4-Dichlorophenoxyacetic Acid**

**Solution:** Add 2,4-dichlorophenoxyacetic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except 2,4-dichlorophenoxyacetic acid solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile 2,4-dichlorophenoxyacetic acid solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of bacteria that can utilize 2,4-dichlorophenoxyacetic acid as sole carbon source.

**Mineral Medium with 2,4-Dichlorotoluene****Composition** per liter:

NH <sub>4</sub> Cl .....	0.5g
Yeast extract .....	0.1g
2,4-Dichlorotoluene .....	0.1g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	670.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	340.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	112.0mg
CaCl <sub>2</sub> .....	14.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.5mg
FeCl <sub>3</sub> .....	0.13mg
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of 2,4-dichlorotoluene-degrading *Pseudomonas* species.

**Mineral Medium H-3****Composition** per liter:

Agar (if needed) .....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.3g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaHCO <sub>3</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate solution .....	20.0mL
Trace elements solution .....	5.0mL

**Ferric Ammonium Citrate Solution:****Composition** per 20.0mL:

Ferric ammonium citrate .....	0.05g
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**Preparation of Ferric Ammonium Citrate Solution:**

Add ferric ammonium citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
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CoCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.002g

**Preparation of Trace Elements Solution:**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. For chemolithotrophic growth, incubate the culture under an atmosphere of 2% (v/v) O<sub>2</sub>, 10% CO<sub>2</sub>, 60% H<sub>2</sub>, and 28% N<sub>2</sub>.

**Preparation of Medium:**

Add components, except ferric ammonium citrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile ferric ammonium citrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes. Incubate cultures in 60% H<sub>2</sub> + 28% N<sub>2</sub> + 10% CO<sub>2</sub> + 2% O<sub>2</sub>.

**Use:** For the chemolithotrophic growth of *Alcaligenes eutrophus*.

**Mineral Medium for Hydrogen Bacteria****Composition** per liter:

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	2.9g
KH <sub>2</sub> PO <sub>4</sub> .....	2.3g
NH <sub>4</sub> Cl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaHCO <sub>3</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g
Ferric ammonium citrate solution .....	20.0mL

**Ferric Ammonium Citrate Solution:****Composition** per 20.0mL:

Ferric ammonium citrate .....	0.05g
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**Preparation of Ferric Ammonium Citrate Solution:**

Add ferric ammonium citrate to 20.0mL of distilled/deionized water. Filter sterilize.

**Preparation of Medium:**

Add components, except ferric ammonium citrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add the sterile ferric ammonium citrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes. Incubate inoculated medium at 30°C under 60% H<sub>2</sub> + 25% N<sub>2</sub> + 10% CO<sub>2</sub> + 5% O<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Alcaligenes eutrophus*, *Hydrogenophaga flava*, and *Hydrogenophaga pseudoflava*.

**Mineral Medium with 2-Hydroxybiphenyl (DSMZ Medium 465a)****Composition** per 1010mL:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.05g
Hydroxybiphenyl solution .....	10.0mL
Trace elements solution SL-4 .....	1.0mL
pH 7.25 ± 0.2 at 25°C	

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Hydroxybiphenyl Solution:****Composition per 100.0mL:**

2-Hydroxybiphenyl .....	5.0g
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**Preparation of Hydroxybiphenyl Solution:** Add 2-hydroxybiphenyl to 100.0mL ethanol. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except hydroxybiphenyl solution, to 1.0L distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add hydroxybiphenyl solution to a sterile culture vessel so that the final concentration of 2-hydroxybiphenyl will be 0.5g/L medium. Let the ethanol evaporate under sterile conditions. Aseptically add the liquid medium to the culture vessel to achieve the appropriate concentration of biphenyl.

**Use:** For the cultivation of *Pseudomonas* sp. and other hydroxybiphenyl-utilizing bacteria.

**Mineral Medium, Nagel and Andreesen****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
NaCl .....	0.05g
CaCl <sub>2</sub> .....	0.01g
MnSO <sub>4</sub> .....	0.01g
Phosphate solution .....	50.0mL
Vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.5 ± 0.2 at 25°C

**Phosphate Solution:****Composition per 50.0mL:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	1.45g
KH <sub>2</sub> PO <sub>4</sub> .....	0.25g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition per liter:**

Folic acid .....	20.0g
α-Lipoic acid .....	50.0mg
p-Aminobenzoic acid .....	50.0mg
Pantothenic acid .....	50.0mg
Riboflavin .....	50.0mg

Thiamine·HCl .....	50.0mg
Vitamin B <sub>12</sub> .....	50.0mg
Nicotine amide .....	25.0mg
Biotin .....	20.0mg
Nicotinic acid .....	20.0mg
Pyridoxamine·HCl .....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Stir for 2 hr. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except phosphate solution, vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 944.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 50.0mL of sterile phosphate solution 5.0mL of sterile vitamin solution and 1.0mL of sterile trace elements solution SL-10. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Arthrobacter* species, *Mycobacterium* species, *Paracoccus denitrificans*, *Pseudomonas putida*, and *Rhodococcus* species.

**Mineral Medium with Naphthalene****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
Ferric ammonium citrate .....	5.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	3.0g
NH <sub>4</sub> Cl .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Titriplex I .....	10.0mg
CoSO <sub>4</sub> .....	10.0μg
Naphthalene crumbs .....	variable

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except naphthalene crumbs, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Inoculate tubes or flasks and place in a dessicator jar in which crumbs of naphthalene will be evaporated.

**Use:** For the cultivation of bacteria that can utilize naphthalene as sole carbon source.

**Mineral Medium with *o*-Nitrophenol (DSMZ Medium 461c)****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
NaCl .....	0.05g

CaCl <sub>2</sub> .....	0.01g
MnSO <sub>4</sub> .....	0.01g
<i>o</i> -Nitrophenol solution.....	200.0mL
Phosphate solution.....	100.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	7.7mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Phosphate Solution:****Composition 100mL:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	1.45g
KH <sub>2</sub> PO <sub>4</sub> .....	0.25g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

***o*-Nitrophenol Solution:****Composition liter:**

<i>o</i> -Nitrophenol.....	0.5g
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**Preparation of *o*-Nitrophenol Solution:** Dissolve *o*-nitrophenol in phosphate buffer (50 mM, pH 7.5) and bring volume to 1.0L. Sterilize by filtration.

**Preparation of Medium:** Add components, except phosphate solution and *o*-nitrophenol solution, to distilled/deionized water and bring volume to 700.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 100.0mL phosphate solution and 200.0mL *o*-nitrophenol solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *o*-nitrophenol-utilizing bacteria.

**Mineral Medium with PCP  
(DSMZ Medium 465b)**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O.....	0.05g
Pentachlorophenol solution.....	100.0mL
Trace elements solution SL-4.....	1.0mL
pH 7.25 ± 0.2 at 25°C	

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Pentachlorophenol Solution:****Composition per liter:**

Pentachlorophenol.....	1.0g
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**Preparation of Pentachlorophenol Solution:** Add pentachlorophenol to 1.0L 0.1M NaOH. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except pentachlorophenol solution, to 900.0mL distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL sterile pentachlorophenol solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Sphingobium chlorophenolicum*=*Sphingomonas chlorophenolica* and other pentachlorophenol-utilizing bacteria.

**Mineral Medium, pH 7.25**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O.....	0.05g
Trace elements solution SL-4.....	1.0mL
pH 7.25 ± 0.2 at 25°C	

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Trace elements solution SL-6.....	100.0mL

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Azotobacter* species, *Pseudomonas* species, and *Sphingomonas* species.

### Mineral Medium with Phenol

**Composition** per liter:

Phenol .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaCl .....	0.5g
CaCl <sub>2</sub> .....	0.02g
FeSO <sub>4</sub> .....	0.02g
Wolfe's mineral solution .....	10.0mL

### Wolfe's Mineral Solution:

**Composition** per liter

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriiloacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitriiloacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except phenol, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add the phenol. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudomonas putida*.

### Mineral Medium with Phenylacetate

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
Ferric ammonium citrate .....	5.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	3.0g
NH <sub>4</sub> Cl .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Titriplex I .....	10.0mg
CoSO <sub>4</sub> .....	10.0μg
Phenylacetic acid solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

### Phenylacetic Acid Solution:

**Composition** per 10.0mL:

Phenylacetic acid .....	0.5g
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**Preparation of Phenylacetic Acid Solution:** Add phenylacetic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except phenylacetic acid solution, to distilled/deionized water and

bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile phenylacetic acid solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of bacteria that can utilize phenylacetic acid as sole carbon source.

### Mineral Medium with Pyrrolic Acid (DSMZ Medium 461b)

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
NaCl .....	0.05g
CaCl <sub>2</sub> .....	0.01g
MnSO <sub>4</sub> .....	0.01g
Phosphate solution .....	100.0mL
Pyrrolic acid solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Phosphate Solution:

**Composition** 100mL:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	1.45g
KH <sub>2</sub> PO <sub>4</sub> .....	0.25g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Pyrrolic Acid Solution:

**Composition** liter:

Pyrrolic acid .....	10.0g
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**Preparation of Pyrrolic Acid Solution:** Dissolve pyrrolic acid in phosphate buffer (50 mM, pH 7.5) and bring volume to 1.0L. Sterilize by filtration.

**Preparation of Medium:** Add components, except phosphate solution and pyrrolic acid solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 100.0mL phosphate solution and 10.0mL pyrrolic acid solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of pyrrolic acid-utilizing bacteria.

### Mineral Medium with Quinoline (DSMZ Medium 461a)

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.3g



NaCl .....	0.05g
CaCl <sub>2</sub> .....	0.01g
MnSO <sub>4</sub> .....	0.01g
Phosphate solution .....	100.0mL
Quinoline emulsion .....	100.0mL
Vitamin solution .....	5.0mL
Trace elements solution. SL-10 .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
H <sub>3</sub> BO <sub>3</sub> .....	300.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	7.7mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Vitamin Solution:****Composition per liter:**

Vitamin B <sub>12</sub> .....	50.0mg
Pantothenic acid .....	50.0mg
Riboflavin .....	50.0mg
Alpha-lipoic acid .....	50.0mg
p-Aminobenzoic acid .....	50.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	50.0mg
Nicotinic acid .....	25.0mg
Nicotine amide .....	25.0mg
Biotin .....	20.0mg
Folic acid .....	20.0mg
Pyridoxamine-HCl .....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Phosphate Solution:****Composition 100mL:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	1.45g
KH <sub>2</sub> PO <sub>4</sub> .....	0.25g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Quinoline Emulsion:****Composition liter:**

Quinoline .....	3.0g
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**Preparation of Quinoline Emulsion:** Prepare an emulsion of quinoline in 50 mM phosphate buffer by stirring or ultrasonication. Add quinoline acid to phosphate buffer (50 mM, pH 7.5) and bring volume to 1.0L. Stir vigorously or sonicate to form emulsion. Autoclave or 15 min at 15 psi pressure–121°C in a gas tight vessel. Cool to room temperature.

**Preparation of Medium:** Add components, except phosphate solution, vitamin solution, and quinoline emulsion to distilled/deionized water and bring volume to 795.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add

100.0mL phosphate solution, 5.0mL vitamin solution, and 100.0mL quinoline emulsion. Mix thoroughly. Aseptically distribute into sterile tubes or flasks

**Use:** For the cultivation of *Rhodococcus rhodochrous* (*Rhodococcus roseus*) and other quinoline-utilizing bacteria.

**Mineral Medium with Salicylate****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
Ferric ammonium citrate .....	5.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	3.0g
NH <sub>4</sub> Cl .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Titriplex I .....	10.0mg
CoSO <sub>4</sub> .....	10.0μg
Salicylic acid solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Salicylic Acid Solution:****Composition per 10.0mL:**

Salicylic acid .....	0.5g
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**Preparation of Salicylic Acid Solution:** Add salicylic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except salicylic acid solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile salicylic acid solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of bacteria that can utilize salicylic acid as sole carbon source.

**Mineral Medium with Sulfobenzoic Acid  
(DSMZ Medium 465e)****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.05g
2-Sulfobenzoic acid .....	50.0mL
Vitamin solution .....	2.5mL
Trace elements solution SL-4 .....	1.0mL

pH 7.25 ± 0.2 at 25°C

**Trace Elements Solution SL-4:****Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

**Composition per 100mL:**

Pyridoxamine .....	5.0mg
Vitamin B <sub>12</sub> .....	2.0mg
Nicotinic acid .....	2.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	1.0mg
<i>p</i> -Aminobenzoate .....	1.0mg
Ca-pantothenate .....	0.5mg
Biotin .....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### 2-Sulfobenzoic Acid Solution:

**Composition per 100.0mL:**

2-Sulfobenzoic acid .....	2.0g
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**Preparation of 2-Sulfobenzoic Acid Solution:** Add 2-sulfobenzoic acid to 100.0mL distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution and 2-sulfobenzoic acid solution, to 947.5mL distilled/deionized water. Adjust pH to 7.25. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 2-sulfobenzoic acid solution and vitamin solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of sulfobenzoic acid-utilizing bacteria.

#### Mineral Medium with Toluene

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
Ferric ammonium citrate .....	5.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	3.0g
NH <sub>4</sub> Cl .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Titriplex I .....	10.0mg
CoSO <sub>4</sub> .....	10.0μg
Toluene .....	1 drop

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except toluene, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Inoculate tubes or flasks and place in a dessicator jar in which 1 drop of toluene will be evaporated.

**Use:** For the cultivation of bacteria that can utilize toluene as sole carbon source.

#### Mineral Medium with Vitamins

**Composition per 1002.5mL:**

Mineral medium .....	1000.0mL
Schlegel's vitamin solution .....	2.5mL

#### Mineral Medium:

**Composition per 1010.0mL:**

Na <sub>2</sub> HPO <sub>4</sub> .....	2.44g
KH <sub>2</sub> PO <sub>4</sub> .....	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements solution SL-4 .....	10.0mL
pH 6.9 ± 0.2 at 25°C	

#### Trace Elements Solution SL-4:

**Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

#### Trace Elements Solution SL-6:

**Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Schlegel's Vitamin Solution:

**Composition per 100.0mL:**

Nicotinic acid .....	2.0g
Pyridoxamine .....	5.0mg
Cyanocobalamin .....	2.0mg
<i>p</i> -Aminobenzoate .....	1.0mg
Thiamine .....	1.0mg
Calcium DL-pantothenate .....	0.5mg
Biotin .....	0.2mg

**Preparation of Schlegel's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Filter sterilize.

**Preparation of Medium:** Add components, except Schlegel's vitamin solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 2.5mL of sterile Schlegel's vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Pseudomonas chlororaphis* and *Rhodococcus* species.

#### Mineral Salts Agar

**Composition per liter:**

Agar .....	15.0g
NaNO <sub>3</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
MgSO <sub>4</sub> .....	0.5g
KCl .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14g
Yeast extract .....	0.02g
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·H <sub>2</sub> O .....	0.01g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position. Add a strip of sterile filter paper onto cooled slant. Inoculate organisms on filter paper.

**Use:** For the cultivation and maintenance of *Cytophaga aurantiaca* and *Sporocytophaga myxococcoides*.

#### Mineral Salts Enrichment Medium

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	1.36g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0mg
MnSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.5mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	2.5mg
Na <sub>2</sub> HPO <sub>4</sub> .....	2.13mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enrichment and cultivation of *Caulobacter* species.

#### Mineral Salts Medium

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Ferric ammonium citrate.....	5.0mg
Modified Hoagland trace elements solution.....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Modified Hoagland Trace Elements Solution:

**Composition** per 3.6L:

H <sub>3</sub> BO <sub>3</sub> .....	11.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	7.0g
AlCl <sub>3</sub> .....	1.0g
CoCl <sub>2</sub> .....	1.0g
CuCl <sub>2</sub> .....	1.0g
KI.....	1.0g
NiCl <sub>2</sub> .....	1.0g
ZnCl <sub>2</sub> .....	1.0g
BaCl <sub>2</sub> .....	0.5g
KBr.....	0.5g
LiCl.....	0.5g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.5g
SeCl <sub>4</sub> .....	0.5g
SnCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.5g
NaVO <sub>3</sub> ·H <sub>2</sub> O.....	0.1g

#### Preparation of Modified Hoagland Trace Elements

**Solution:** Prepare each component as a separate solution. Dissolve each salt in approximately 100.0mL of distilled/deionized water. Adjust the pH of each solution to below 7.0. Combine all the salt solutions and bring the volume to 3.6L with distilled/deionized water. Adjust the pH to 3–4. A yellow precipitate may form after mixing. After a few days, it will turn into a fine white precipitate. Mix the solution thoroughly before using.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodococcus rhodochrous*.

#### Mineral Salts Medium with Butane

**Composition** per liter of tap water:

(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	8.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Yeast extract.....	100.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	60.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	60.0μg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	15.0μg

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Incubate inoculated medium in 88% air + 7% *n*-butane + 5% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Pseudomonas butanovora*.

#### Mineral Salts Medium with Methanol

**Composition** per liter:

NaNH <sub>4</sub> HPO <sub>4</sub> ·4H <sub>2</sub> O.....	1.74g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O.....	0.54g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
KCl.....	0.04g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0mg
Methanol.....	5.0mL
Trace mineral solution.....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Trace Mineral Solution:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.08g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.06g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	25.0mg

**Preparation of Trace Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Filter sterilize methanol. Aseptically add sterile methanol to cooled, sterile basal medium.

**Use:** For the cultivation and maintenance of *Rhodococcus rhodochrous*.

#### Mineral Salts Medium with Methanol and Yeast Extract

**Composition** per liter:

NaNH <sub>4</sub> HPO <sub>4</sub> ·4H <sub>2</sub> O.....	1.74g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O.....	0.54g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Yeast extract.....	0.2g
KCl.....	0.04g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0mg
Methanol.....	5.0mL
Trace mineral solution.....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Trace Mineral Solution:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
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MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.08g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.06g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	25.0mg

**Preparation of Trace Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Filter sterilize methanol. Aseptically add sterile methanol to cooled, sterile basal medium. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

#### Mineral Salts Medium for Thermophiles

**Composition per liter:**

NaNO <sub>3</sub> .....	0.25g
NH <sub>4</sub> Cl .....	0.25g
Na <sub>2</sub> HPO <sub>4</sub> .....	210.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	200.0mg
NaH <sub>2</sub> PO <sub>4</sub> .....	90.0mg
KCl .....	40.0mg
CaCl <sub>2</sub> .....	15.0mg
FeSO <sub>4</sub> .....	1.0mg
Trace minerals solution .....	10.0mL
<i>n</i> -Heptadecane .....	1.0mL

#### Trace Minerals Solution:

**Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
MoO <sub>3</sub> .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	500.0μg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	18.0μg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	7.0μg

**Preparation of Trace Minerals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus thermoleovorans*.

#### Mineral Salts Peptonized Milk Agar (SPMA)

**Composition per liter:**

Agar .....	15.0g
Milk, peptonized .....	1.0g
Mineral solution .....	100.0mL

#### Mineral Solution:

**Composition per 100.0mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g
MnCl <sub>2</sub> .....	0.1mg

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except mineral solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile mineral solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of freshwater *Myxobacterium* species.

#### Mineral Salts for Thermophiles

##### (L Salts for Thermophiles)

**Composition per liter:**

NaNO <sub>3</sub> .....	0.25g
NH <sub>4</sub> Cl .....	0.25g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.09g
KCl .....	0.04g
CaCl <sub>2</sub> .....	0.02g
FeSO <sub>4</sub> .....	1.0mg
Trace mineral solution .....	10.0mL
<i>n</i> -Heptadecane .....	1.0mL

#### Trace Mineral Solution:

**Composition per liter:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
MoO <sub>3</sub> .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	500.0μg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	18.0μg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	7.0μg

**Preparation of Trace Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except *n*-heptadecane, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add the *n*-heptadecane. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Bacillus thermoleovorans*.

#### Minerals Modified Medium

**Composition per liter:**

Lactose .....	20.0g
Sodium glutamate .....	12.7g
NH <sub>4</sub> Cl .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.8g
Sodium formate .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
L-Aspartic acid .....	0.048g
L-Cystine .....	0.04g
L-Arginine .....	0.04g
Ferric ammonium citrate .....	0.02g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
Bromocresol Purple .....	0.02g
Thiamine .....	2.0mg
Nicotinic acid .....	2.0mg
Pantothenic acid .....	2.0mg

pH 6.7 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 800.0mL. Add remaining components and bring volume to 1.0L. Mix thoroughly. Ad-

just pH to 6.7. Distribute into tubes or flasks. Autoclave for 10 min at 10 psi pressure–116°C. Check pH after autoclaving. This medium is double strength.

**Use:** For the enumeration of coliform bacteria in water.

### Minimal Medium for Denitrifying Bacteria

**Composition** per liter:

Solution A .....	980.0mL
Solution B .....	10.0mL
Solution C .....	10.0mL

#### Solution A:

**Composition** per 980.0mL:

KNO <sub>3</sub> .....	5.0g
Carbon source .....	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.87g
KH <sub>2</sub> PO <sub>4</sub> .....	0.54g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution B:

**Composition** per 100.0mL:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
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**Preparation of Solution B:** Add MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution C:

**Composition** per 100.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.05g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
HCl (0.1N solution) .....	100.0mL

**Preparation of Solution C:** Combine components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Aseptically combine 980.0mL of cooled sterile solution A, 10.0mL of cooled sterile solution B, and 10.0mL of cooled sterile solution C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of denitrifying bacteria.

### Mixotrophic *Nitrobacter* Medium (DSMZ Medium 756a)

**Composition** per liter:

NaNO <sub>2</sub> .....	2.0g
Yeast extract .....	1.5g
Peptone .....	1.5g
Na-pyruvate .....	0.55g
Stock solution .....	100.0mL
Trace elements solution .....	1.0mL

pH 7.4 ± 0.2 at 25°C

#### Stock Solution:

**Composition** per liter:

NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	97.3mg
H <sub>3</sub> BO <sub>3</sub> .....	49.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	43.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	37.1mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	33.8mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	25.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Nitrobacter vulgaris*.

### Mixotrophic *Nitrobacter* Medium (LMG Medium 246)

**Composition** per liter:

NaNO <sub>2</sub> .....	2.0g
Yeast extract .....	1.50g
Peptone .....	1.50g
Na-pyruvate .....	0.55g
Stock solution .....	100.0mL
Trace elements solution .....	1.0mL

pH 7.4 ± 0.2 at 25°C

#### Stock Solution:

**Composition** per liter:

NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

**Composition** per liter:

(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .....	437.10mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	97.30mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	43.10mg
H <sub>3</sub> BO <sub>3</sub> .....	39.40mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	33.80mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	25.00mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4 with NaOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of mixotrophic *Nitrobacter* spp.

### Mixotrophic *Nitrobacter* Medium, 10% (DSMZ Medium 756b)

**Composition** per liter:

NaNO <sub>2</sub> .....	2.0g
Yeast extract .....	0.15g
Peptone .....	0.15g
Na-pyruvate .....	0.055g

Stock solution.....	100.0mL
Trace elements solution .....	1.0mL
pH 8.6 ± 0.2 at 25°C	

**Stock Solution:****Composition per liter:**

NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:****Composition per liter:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	97.3mg
H <sub>3</sub> BO <sub>3</sub> .....	49.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	43.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	37.1mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	33.8mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	25.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.6. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Nitrobacter hamburgensis* and *Nitrobacter vulgaris*.

### ML Medium (Minimal Lactate Medium)

**Composition per liter:**

Sodium lactate .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
L-Cysteine .....	0.5g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg
NaHCO <sub>3</sub> solution .....	25.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	25.0mL
pH 6.8 ± 0.2 at 25°C	

**NaHCO<sub>3</sub> Solution:****Composition per 25.0mL:**

NaHCO <sub>3</sub> .....	4.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize. Gas with O<sub>2</sub>-free 97% N<sub>2</sub> + 3% H<sub>2</sub>. Cap with a rubber stopper.

**FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:****Composition per 25.0mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.0mg
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**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize. Gas with O<sub>2</sub>-free 97% N<sub>2</sub> + 3% H<sub>2</sub>. Cap with a rubber stopper.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution and FeSO<sub>4</sub>·7H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling under O<sub>2</sub>-free 97% N<sub>2</sub> + 3% H<sub>2</sub>. Adjust pH

to 6.8. Continue boiling until resazurin becomes colorless, indicating reduction. Distribute anaerobically under O<sub>2</sub>-free 97% N<sub>2</sub> + 3% H<sub>2</sub> into tubes in 10.0mL volumes. Cap with rubber stoppers. Place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Prior to inoculation, add 0.25mL of sterile NaHCO<sub>3</sub> solution and 0.25mL of sterile FeSO<sub>4</sub>·7H<sub>2</sub>O solution to each test tube containing 10.0mL of sterile basal medium.

**Use:** For the cultivation and maintenance of *Desulfovibrio* species.

**MMA Salts Medium****Composition per liter:**

Agar, noble .....	20.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.62g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.05g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	70.0μg
H <sub>3</sub> BO <sub>3</sub> .....	10.0μg
MnSO <sub>4</sub> ·5H <sub>2</sub> O .....	10.0μg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	10.0μg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.0μg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	5.0μg
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.0mg
Monomethylamine solution .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Monomethylamine Solution:****Composition per 10.0mL:**

Monomethylamine .....	1.0g
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**Preparation of Monomethylamine Solution:** Add monomethylamine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except monomethylamine solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL of sterile methylamine solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Hyphomicrobium* species and *Methylophilus methylotrophus*.

**MMB Medium****Composition per liter:**

Glucose .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.25g
Peptone .....	0.15g
Yeast extract .....	0.15g
Glucose solution .....	20.0mL
Hutner's basal salts solution .....	20.0mL
Vitamin solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

**Glucose Solution:****Composition per 20.0mL:**

Glucose .....	1.5g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Hutner's Basal Salts Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitilotriacetic acid .....	10.0g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>7</sub> ·O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44" .....	50.0mL

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Vitamin Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	0.01mg
Calcium DL-pantothenate .....	0.5mg
Nicotinamide .....	0.5mg
Pyridoxine-HCl .....	0.5mg
Riboflavin .....	0.5mg
Thiamine-HCl .....	0.5mg
Biotin .....	0.2mg
Folic acid .....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and vitamin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile glucose solution and 20.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Angulomicrobium tetradrale*, *Labrys monachus*, *Prosthecomicrobium polysphaeroidum*, and *Aquabacter spiritensis*.

**MMS Medium for *Thermotoga neapolitana*****Composition per liter:**

NaCl .....	6.93g
Starch .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.38g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.38g
KCl .....	0.16g
NaBr .....	25.0mg
H <sub>3</sub> BO <sub>3</sub> .....	7.5mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.8mg
(NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> ) <sub>2</sub> .....	2.0mg
Resazurin .....	1.0mg

KI .....	0.025mg
Wolfe's mineral solution .....	15.0mL
pH 6.5 ± 0.2 at 25°C	

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitriloacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thermotoga neapolitana*.

**MN Marine Medium****Composition per liter:**

Noble agar .....	10.0g
NaNO <sub>3</sub> .....	0.75g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	3.0mg
Ferric ammonium citrate .....	3.0mg
Disodium potassium EDTA .....	0.5mg
Trace metals A-5 .....	1.0mL

pH 8.5 ± 0.2 at 25°C

**A-5 Trace Metal Mix:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.039g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of A-5 Trace Metal Mix:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to 750.0mL of seawater and bring volume to 1L with glass-distilled water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. After autoclaving, adjust pH to 8.5 with KOH.

**Use:** For the cultivation and maintenance of marine cyanobacteria.

**MN Marine Medium with Vitamin B<sub>12</sub>****Composition per liter:**

Noble agar .....	10.0g
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NaNO <sub>3</sub> .....	0.75g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.02g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid.....	3.0mg
Ferric ammonium citrate.....	3.0mg
Disodium potassium EDTA.....	0.5mg
Vitamin B <sub>12</sub> .....	20.0μg
Trace metals A-5.....	1.0mL
pH 8.5 ± 0.2 at 25°C	

**A-5 Trace Metal Mix:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.079g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.039g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.049g

**Preparation of A-5 Trace Metal Mix:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to 750.0mL of seawater and bring volume to 1.0L with glass-distilled water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. After autoclaving, adjust pH to 8.5 with KOH.

**Use:** For the cultivation and maintenance of *Dermocarpa* species, *Dermocarpella* species, *Myxosarcina* species, *Phormidium* species, *Pleurocapsa* species, *Synechococcus* species, *Synechocystis* species, and *Xenococcus* species.

**Moderate Halophilic Medium (HM)****Composition per liter:**

NaCl.....	81.0g
Agar.....	20.0g
Yeast extract.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	9.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	7.0g
Proteose peptone No. 3.....	5.0g
KCl.....	2.0g
Glucose.....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.36g
NaHCO <sub>3</sub> .....	0.06g
NaBr.....	0.026g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus halophilus*, *Halococcus saccharolyticus*, *Marinococcus albus*, *Marinococcus halophilus*, and *Marinococcus hispanicus*.

**Modified PYNFH Medium****Composition per liter:**

Peptone.....	10.0g
Yeast extract.....	10.0g
Yeast nucleic acid.....	1.0g
Folic acid.....	15.0mg
Hemin.....	1.0mg
Fetal bovine serum, heat inactivated.....	100.0mL
Buffer solution.....	20.0mL
pH 6.5 ± 0.2 at 25°C	

**Buffer Solution:****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	25.0g
KH <sub>2</sub> PO <sub>4</sub> .....	18.1g

**Preparation of Buffer Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except buffer solution and fetal bovine serum, to distilled/deionized water and bring volume to 880.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 20.0mL of sterile buffer solution and 100.0mL of sterile, heat-inactivated fetal bovine serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Dexiostoma campyla*, *Hartmannella vermiformis*, *Naegleria australiensis*, *Naegleria fowleri*, *Naegleria gruberi*, *Naegleria jadini*, *Phytomonas davidi*, *Tetrahymena* species, *Vahlkampfia avara*, and *Wilaertia magna*.

**Modified Semisolid Rappaport-Vassiliadis Medium (MSRV Medium)****Composition per liter:**

MgCl <sub>2</sub> , anhydrous.....	10.93g
NaCl.....	7.34g
Casein hydrolysate.....	4.59g
Tryptose.....	4.59g
Agar.....	2.7g
KH <sub>2</sub> PO <sub>4</sub> .....	1.47g
Malachite Green oxalate.....	0.037g
Novobiocin.....	10.0mL
pH 5.2 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Novobiocin Solution:****Composition per 10.0mL:**

Novobiocin.....	0.02g
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**Preparation of Novobiocin Solution:** Add novobiocin to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except novobiocin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat to boiling. Do not autoclave. Cool to 45°–50°C. Aseptically add 10.0mL of sterile novobiocin solution. Mix thoroughly. Pour into sterile Petri dishes. Air-dry plates for at least 1 hr.

**Use:** For the isolation and cultivation of motile *Salmonella* species from environmental samples.

**Modified Shieh Agar (LMG Medium 215)****Composition per liter:**

Agar.....	15.0g
Peptone.....	5.0g
Yeast extract.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
KH <sub>2</sub> PO <sub>4</sub> .....	50.0mg
NaHCO <sub>3</sub> .....	50.0mg
Na-acetate.....	10.0mg
BaCl <sub>2</sub> ·H <sub>2</sub> O.....	10.0mg



CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	6.7 mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flavobacterium* spp., *Flexibacter* spp., *Chitinophaga pinensis*, and *Flectobacillus major*.

#### **Modified Thermus Medium (DSMZ Medium 630)**

##### **Composition per liter:**

Agar .....	28.0g
Yeast extract .....	2.5g
Tryptone .....	2.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	200.0mg
Nitrilotriacetic acid .....	100.0mg
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg
Phosphate solution .....	100.0mL
Ferric citrate solution .....	0.5mL
Trace elements solution .....	0.5mL
pH 7.2 ± 0.2 at 25°C	

##### **Phosphate Solution:**

##### **Composition liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	43.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.44g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

##### **Ferric Citrate Solution:**

##### **Composition liter:**

Ferric citrate .....	2.5g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

##### **Trace Elements Solution:**

##### **Composition per liter:**

Nitrilotriacetic acid .....	12.8g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.3g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	50.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
H <sub>3</sub> BO <sub>3</sub> .....	20.0mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 7.0 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly. Adjust pH to 6.8.

**Preparation of Medium:** Add components, except phosphate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 100.0mL phosphate solution. Mix thoroughly. Adjust pH to 7.2. Pour into Petri dishes or aseptically distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermus* sp., *Rhodothermus marinus*, and *Albidovulum inexpectatum*.

#### **Modified VWM Medium (DSMZ Medium 503a)**

##### **Composition per 1013.4mL:**

Solution A .....	940.0mL
Solution E .....	50.0mL
Solution F .....	10.0mL
Solution G .....	10.0mL
Solution B .....	1.0mL
Solution C .....	1.0mL
Solution D .....	1.0mL
Solution H .....	0.4mL
pH 7.3 ± 0.2 at 25°C	

##### **Solution A:**

##### **Composition per 940.0mL:**

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

##### **Solution B:**

##### **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

##### **Solution C:**

##### **Composition per liter:**

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
p-Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

##### **Solution D:**

##### **Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Solution E:****Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution F:****Composition** per 10mL:

Taurine ..... 2.5g

**Preparation of Solution F:** Add taurine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution G:****Composition** per 10mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.125g

**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution H:****Composition** per 10mL:

Na-dithionite ..... 0.5g

**Preparation of Solution H:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Sequentially add 1.0mL solution B, 1.0mL solution C, 1.0mL solution D, 50.0mL solution E, 10.0mL solution F, 10.0mL solution G, and 0.4mL solution H, to 940.0mL solution A. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. The pH should be 7.2–7.4.

**Use:** For the cultivation of *Desulfonisporea thiosulfatigenes*.

**Møller Decarboxylase Broth****Composition** per liter:

Amino acid ..... 10.0g  
 Peptic digest of animal tissue ..... 5.0g  
 Beef extract ..... 5.0g  
 Glucose ..... 0.5g  
 Bromcresol Purple ..... 0.01g  
 Cresol Red ..... 5.0mg  
 Pyridoxal ..... 5.0mg

pH 6.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Use L-lysine, L-arginine, or L-ornithine. Mix thoroughly. Gently heat until dissolved. Distribute into screw-capped tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. A slight precipitate may form in the ornithine broth.

**Use:** For the differentiation of Gram-negative enteric bacteria based on the production of arginine dihydrolase, lysine decarboxylase, or ornithine decarboxylase.

**Møller KCN Broth Base****Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub> ..... 5.64g  
 NaCl ..... 5.0g  
 Pancreatic digest of casein ..... 1.5g  
 Peptic digest of animal tissue ..... 1.5g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.225g  
 KCN solution ..... 0.15mL

pH 7.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**KCN Solution:****Composition** per 100.0mL:

KCN ..... 0.5g

**Preparation of KCN Solution:** Add KCN to 100.0mL of cold distilled/deionized water. Mix thoroughly and cap. Do not mouth pipette.

**Caution:** Cyanide is toxic.

**Preparation of Medium:** Add components, except KCN solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Prior to use, add 0.15mL of KCN solution. Mix thoroughly. Aseptically distribute into sterile tubes.

**Use:** For the differentiation of Gram-negative enteric bacteria on the basis of their ability to grow in the presence of cyanide.

**Moraxella Medium  
(LMG Medium 204)****Composition** per liter:

Special peptone ..... 23.0g  
 Agar ..... 15.0g  
 Glucose ..... 5.0g  
 NaCl ..... 5.0g  
 Soluble starch ..... 1.0g  
 Cysteine hydrochloride ..... 0.3g  
 Sheep blood, sterile defibrinated ..... 50.0mL

pH 7.1 ± 0.2 at 25°C

**Source:** Special peptone is available from Oxoid Unipath.

**Preparation of Medium:** Add components, except sheep blood, to 950.0mL distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 50.0mL sterile sheep blood. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Moraxella osloensis*, *Moraxella atlantae*, and *Cellulomonas hominis*.

**MP Agar****Composition** per liter:

Agar ..... 15.0g  
 Sodium acetate ..... 0.1g  
 Basal medium ..... 1.0L  
 Sodium sulfide solution ..... 3.0mL

pH 7.0–7.5 at 25°C

**Basal Medium:****Composition** per liter:

CaSO<sub>4</sub>·2H<sub>2</sub>O (saturated solution) ..... 20.0mL  
 NH<sub>4</sub>Cl (4% solution) ..... 5.0mL  
 Trace elements solution ..... 5.0mL

MgSO <sub>4</sub> ·7H <sub>2</sub> O (1% solution) .....	1.0mL
K <sub>2</sub> HPO <sub>4</sub> (1% solution) .....	1.0mL

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution:

**Composition** per liter:

Ferrous EDTA solution .....	20.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.1% solution) .....	10.0mL
MnSO <sub>4</sub> ·4H <sub>2</sub> O (0.02% solution) .....	10.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.0005% solution) .....	10.0mL
H <sub>3</sub> BO <sub>3</sub> (0.1% solution) .....	10.0mL
Co(NO <sub>3</sub> ) <sub>2</sub> or CoCl <sub>2</sub> ·6H <sub>2</sub> O (0.01% solution) .....	10.0mL
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O (0.01% solution) .....	10.0mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Ferrous EDTA Solution:

**Composition** per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
EDTA .....	2.0g
HCl, concentrated .....	1.0mL

**Preparation of Ferrous EDTA Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Sodium Sulfide Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Sodium Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Prepare freshly.

**Preparation of Medium:** Add sodium acetate and agar to 1.0L of basal medium. Mix thoroughly. Adjust pH to 7.0–7.5. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 3.0mL of sterile sodium sulfide solution immediately prior to dispensing. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile screw-capped tubes.

**Use:** For the isolation and cultivation of *Beggiatoa* species and myxotrophic strains of *Thiothrix* species from water and environmental sources.

### MP 5 Medium (Mineral Pectin 5 Medium)

**Composition** per liter:

Agar solution .....	500.0mL
Basal medium .....	250.0mL
Mineral solution .....	250.0mL

pH 5.0–6.0 at 25°C

#### Agar Solution:

**Composition** per 500.0mL:

Agar .....	15.0g
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**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Basal Medium:

**Composition** per 250.0mL:

Na <sub>2</sub> HPO <sub>4</sub> .....	6.0g
Pectin, citrus or apple .....	5.0g

KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
NH <sub>4</sub> SO <sub>4</sub> .....	2.0g
Yeast extract .....	1.0g

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Gently heat and bring to boiling.

#### Mineral Solution:

**Composition** per 250.0mL:

FeSO <sub>4</sub> (0.1% solution) .....	1.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O (20% solution) .....	1.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O (0.1% solution) .....	1.0mL
H <sub>3</sub> BO <sub>3</sub> (0.001% solution) .....	1.0mL
MnSO <sub>4</sub> ·H <sub>2</sub> O (0.001% solution) .....	1.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.007% solution) .....	1.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.005% solution) .....	1.0mL
MoO <sub>3</sub> (0.001% solution) .....	1.0mL

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly.

**Preparation of Medium:** Combine 250.0mL of basal medium and 250.0mL of mineral solution. Mix thoroughly. Adjust pH to 5.0–6.0 with 1N HCl. Autoclave the basal medium-mineral solution and agar solution separately for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine the two sterile solutions. Mix thoroughly. Pour immediately into sterile Petri dishes to prevent hydrolysis of the agar.

**Use:** For the cultivation of microorganisms that produce polygalactanase.

### MP 7 Medium (Mineral Pectin 7 Medium)

**Composition** per liter:

Basal medium .....	500.0mL
Mineral solution .....	500.0mL

pH 7.2 ± 0.2 at 25°C

#### Basal Medium:

**Composition** per 500.0mL:

Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	6.0g
Pectin, citrus or apple .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
NH <sub>4</sub> SO <sub>4</sub> .....	2.0g
Yeast extract .....	1.0g

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling.

#### Mineral Solution:

**Composition** per 500.0mL:

FeSO <sub>4</sub> (0.1% solution) .....	1.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O (20% solution) .....	1.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O (0.1% solution) .....	1.0mL
H <sub>3</sub> BO <sub>3</sub> (0.001% solution) .....	1.0mL
MnSO <sub>4</sub> ·H <sub>2</sub> O (0.001% solution) .....	1.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.007% solution) .....	1.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.005% solution) .....	1.0mL
MoO <sub>3</sub> (0.001% solution) .....	1.0mL

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Preparation of Medium:** Combine 500.0mL of basal medium and 500.0mL of mineral solution. Mix thoroughly.

Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Pour into sterile Petri dishes.

**Use:** For the cultivation of microorganisms that produce peptate lyase.

### MPH Agar (Milk Protein Hydrolysate Agar)

#### Composition per liter:

Agar .....	15.0g
Casein hydrolysate .....	9.0g
Glucose .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically distribute into sterile tubes. Cool to 43°–45°C before using.

**Use:** For use in the enumeration of bacteria in water.

### MPOB Medium

#### Composition per 1012.0mL:

Disodium fumarate .....	3.2g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	0.53g
KH <sub>2</sub> PO <sub>4</sub> .....	0.41g
NaCl .....	0.3g
NH <sub>4</sub> Cl .....	0.3g
Yeast extract .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.11g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.10g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Selenite-tungstate solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.0–7.2 at 25°C

#### NaHCO<sub>3</sub> Solution:

##### Composition per 20.0mL:

NaHCO <sub>3</sub> .....	4.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Wolfe's Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Selenite-Tungstate Solution:

##### Composition per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile NaHCO<sub>3</sub> solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks. After inoculation, bring culture bottles to 0.7 bar 80% N<sub>2</sub> + 20% CO<sub>2</sub> overpressure.

**Use:** For the cultivation of *Syntrophobacter* species.

### MPSS Broth

#### Composition per liter:

Peptone .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Succinic acid .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O (0.2% solution) .....	1.0mL
MnSO <sub>4</sub> ·H <sub>2</sub> O (0.2% solution) .....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Spirillum volutans*.

### MPY Agar (Maltose Peptone Yeast Extract Medium) (ATCC Medium 518)

#### Composition per liter:

Agar .....	10.0g
Maltose .....	2.0g
Peptone .....	2.0g

Yeast extract .....	1.0g
Potassium phosphate buffer (1M, pH 7.5) .....	10.0mL
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except potassium phosphate buffer, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Filter sterilize potassium phosphate buffer. Aseptically add sterile potassium phosphate buffer to sterile, cooled basal medium. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spirochaeta aurantia*.

### MRVP Broth (Methyl Red Voges-Proskauer Broth)

#### Composition per liter:

Glucose .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.0g
Pancreatic digest of casein .....	3.5g
Peptic digest of animal tissue .....	3.5g
pH 6.9 ± 0.2 at 25°C	

**Source:** Available as a premixed powder from BD Diagnostic Systems and as a prepared medium from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the differentiation of bacteria based on acid production (Methyl Red test) and acetoin production (Voges-Proskauer reaction).

### MRVP Medium (Methyl Red Voges-Proskauer Medium)

#### Composition per liter:

Glucose .....	5.0g
Peptone .....	5.0g
Phosphate buffer .....	5.0g
pH 7.5 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the differentiation of bacteria based on acid production (Methyl Red test) and acetoin production (Voges-Proskauer reaction).

### MS Agar

#### Composition per liter:

Agar .....	15.0g
Peptone .....	1.0g
Yeast extract .....	1.0g
Glucose .....	1.0g
pH 6.8–7.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Runella slithyformis*.

### MS 1 Agar

#### Composition per liter:

Agar .....	15.0g
Seawater .....	1.0L

**Preparation of Medium:** Add agar to 1.0L of natural seawater. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### MS 3 Agar

#### Composition per liter:

Agar .....	15.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Seawater .....	1.0L

**Preparation of Medium:** Add agar to 500.0mL of natural seawater. Mix thoroughly. Gently heat and bring to boiling. In a separate flask, add (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to 500.0mL of natural seawater. Mix thoroughly. Autoclave both solutions separately for 15 min at 15 psi pressure–121°C. Aseptically combine the two sterile solutions. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### MS 4 Agar

#### Composition per liter:

Agar .....	15.0g
Glucose .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Seawater .....	1.0L

**Preparation of Medium:** Add agar to 500.0mL of natural seawater. Mix thoroughly. Gently heat and bring to boiling. Add (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to 250.0mL of natural seawater. Mix thoroughly. Add glucose to 250.0mL of natural seawater. Mix thoroughly. Autoclave the three solutions separately for 15 min at 15 psi pressure–121°C. Aseptically combine the three sterile solutions. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### MS Medium for *Acidiphilium cryptum*

#### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
KCl .....	0.1g
Glucose solution .....	20.0mL
Yeast extract solution .....	10.0mL
pH 3.0 ± 0.2 at 25°C	

#### Glucose Solution:

##### Composition per 100.0mL:

D-Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Yeast Extract Solution:****Composition** per 10.0mL:

Yeast extract ..... 1.0g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution and yeast extract solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 3.0 with 2N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile glucose solution and 10.0mL of sterile yeast extract solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Acidiphilium cryptum*.

**MS Medium for *Leptospirillum* species****Composition** per liter:

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 2.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.25g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.1g  
KCl ..... 0.1g  
FeSO<sub>4</sub> solution ..... 50.0mL  
pH 1.6 ± 0.2 at 25°C

**Fe<sub>2</sub>SO<sub>4</sub> Solution:****Composition** per 10.0mL:

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 4.0g

**Preparation of Fe<sub>2</sub>SO<sub>4</sub> Solution:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except FeSO<sub>4</sub> solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 1.6 with 4N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 50.0mL of sterile FeSO<sub>4</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leptospirillum* species.

**MS Medium for Methanogens****Composition** per 340.0mL:

Agar ..... 8.0g  
NaHCO<sub>3</sub> ..... 2.4g  
L-Cysteine-sulfide reducing agent ..... 16.0mL  
Mineral solution 1 ..... 15.0mL  
Mineral solution 2 ..... 15.0mL  
Sodium formate (20% solution) ..... 6.0mL  
Yeast extract-soybean casein solution ..... 4.0mL  
Sodium acetate (25% solution) ..... 4.0mL  
Wolfe's vitamin solution ..... 4.0mL  
Wolfe's mineral solution ..... 4.0mL  
FeSO<sub>4</sub>·7H<sub>2</sub>O (0.2% solution) ..... 0.4mL  
Resazurin (0.1% solution) ..... 0.4mL  
pH 7.0 ± 0.2 at 25°C

**L-Cysteine-Sulfide Reducing Agent:****Composition** per 400.0mL:

L-Cysteine-HCl·H<sub>2</sub>O ..... 5.0g  
Na<sub>2</sub>S (12.5% solution) ..... 40.0mL  
NaOH (1N solution) ..... 30.0mL

**Preparation of L-Cysteine-Sulfide Reducing Agent:** Add distilled/deionized water to a 500.0mL round-bottomed flask. Add freshly prepared NaOH solution. Gently heat and bring to boiling under 100% N<sub>2</sub>. Remove gas-sing probe. Add L-cysteine-HCl·H<sub>2</sub>O. Add freshly prepared

Na<sub>2</sub>S solution. Renew gassing for several minutes. Cap with rubber stoppers. Distribute into 8.0mL/18.0mm Hungate tubes.

**Mineral Solution 1:****Composition** per liter:

K<sub>2</sub>HPO<sub>4</sub> ..... 6.0g

**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Mineral Solution 2:****Composition** per liter:

NaCl ..... 12.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 6.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 6.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.6g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.16g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Yeast Extract-Soybean Casein Solution:****Composition** per 100.0mL:

Yeast extract ..... 20.0g  
Pancreatic digest of casein ..... 20.0g

**Preparation of Yeast Extract-Soybean Casein Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Wolfe's Mineral Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitriloacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.5g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub> ..... 0.1g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
AlK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.01g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Wolfe's Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Thioctic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 408.0mL. Gently heat and bring to boiling under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue boiling until resazurin turns colorless, indicating reduction.

Adjust pH to 7.0. Anaerobically distribute into tubes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Cap with rubber stoppers and aluminum crimp closures. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Methanobacterium thermoautotrophicum*, *Methanobacterium wolfei*, *Methanobrevibacter smithii*, *Methanogenium bourgense*, and *Methanogenium* species.

#### MS Medium for *Thiobacillus caldus*

##### Composition per liter:

Sulfur, powdered.....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
KCl.....	0.1g
Yeast extract solution.....	10.0mL
pH 3.5 ± 0.2 at 25°C	

**Preparation of Sulfur:** Sterilize powdered elemental sulfur by steaming for 3 hr at 0 psi pressure–100°C on 3 successive days.

##### Yeast Extract Solution:

##### Composition per 10.0mL:

Yeast extract.....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except elemental sulfur and yeast extract solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 3.5 with 1N sulfuric acid. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 5.0g of sterile elemental sulfur and 10.0mL of sterile yeast extract solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus caldus*.

#### MS Medium for *Thiobacillus ferrooxidans*

##### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
KCl.....	0.1g
FeSO <sub>4</sub> solution.....	50.0mL
pH 2.2 ± 0.2 at 25°C	

##### Fe<sub>2</sub>SO<sub>4</sub> Solution:

##### Composition per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	4.0g
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**Preparation of Fe<sub>2</sub>SO<sub>4</sub> Solution:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except FeSO<sub>4</sub> solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 2.2 with 4N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 50.0mL of sterile FeSO<sub>4</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus ferrooxidans*.

#### MS Medium for *Thiobacillus thiooxidans*

##### Composition per liter:

Sulfur, powdered.....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
KCl.....	0.1g

pH 3.5 ± 0.2 at 25°C

**Preparation of Sulfur:** Sterilize powdered elemental sulfur by steaming for 3 hr at 0 psi pressure–100°C on 3 successive days.

**Preparation of Medium:** Add components, except elemental sulfur, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.5 with 1N sulfuric acid. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 5.0g of sterile elemental sulfur. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus thiooxidans*.

#### MSA-Fe Medium

##### Composition per 1010.0mL:

NaCl.....	5.8g
Pancreatic digest of casein.....	2.0g
Yeast extract.....	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
Mercaptoethanesulfonic acid.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O.....	0.4g
Resazurin.....	0.5mg
Trace elements solution.....	10.0mL

pH 7.5 ± 0.2 at 25°C

##### Trace Elements Solution:

##### Composition per 100.0mL:

Sodium EDTA·2H <sub>2</sub> O.....	50.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	15.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	10.0mg
ZnCl <sub>2</sub> .....	10.0mg
AlCl <sub>3</sub> ·6H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	3.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
NiSO <sub>4</sub> ·6H <sub>2</sub> O.....	2.0mg
H <sub>2</sub> SeO <sub>3</sub> .....	1.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with 2N NaOH. Sparge with 100% N<sub>2</sub> for 30 min. Anaerobically distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bacillus infermus*.

#### MSV AcS Agar

##### Composition per liter:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.187g
Sodium acetate.....	0.15g
MSV agar.....	1.0L

pH 7.2–7.5 at 25°C

##### MSV Agar:

##### Composition per liter:

Agar.....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
EDTA .....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	2.0mg
Vitamin mix .....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Mix:

**Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g
Pyridoxine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	0.01g
Coccarboxylase .....	0.01g
Inositol .....	0.01g
Thiamine .....	0.01g
Riboflavin .....	0.01g
Biotin .....	0.5mg
Cyanocobalamin .....	0.5mg
Folic acid .....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar add sodium acetate and Na<sub>2</sub>S·9H<sub>2</sub>O. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

#### MSV Agar

**Composition per liter:**

Agar .....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
EDTA .....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	2.0mg
Vitamin mix .....	1.0mL

pH 7.2–7.5 at 25°C

#### Vitamin Mix:

**Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g
Pyridoxine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	0.01g
Coccarboxylase .....	0.01g
Inositol .....	0.01g
Thiamine .....	0.01g
Riboflavin .....	0.01g
Biotin .....	0.5mg
Cyanocobalamin .....	0.5mg
Folic acid .....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into

tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

#### MSV Broth

**Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
EDTA .....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	2.0mg
Vitamin mix .....	1.0mL

pH 7.2–7.5 at 25°C

#### Vitamin Mix:

**Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g
Pyridoxine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	0.01g
Coccarboxylase .....	0.01g
Inositol .....	0.01g
Thiamine .....	0.01g
Riboflavin .....	0.01g
Biotin .....	0.5mg
Cyanocobalamin .....	0.5mg
Folic acid .....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2–7.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

#### MSV GS Agar

**Composition per liter:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.187g
Glucose .....	0.15g
MSV agar .....	1.0L

pH 7.2–7.5 at 25°C

#### MSV Agar:

**Composition per liter:**

Agar .....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
EDTA .....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	2.0mg
Vitamin mix .....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Mix:

**Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g



Pyridoxine.....	0.01g
<i>p</i> -Aminobenzoic acid.....	0.01g
Coccarboxylase.....	0.01g
Inositol.....	0.01g
Thiamine.....	0.01g
Riboflavin.....	0.01g
Biotin.....	0.5mg
Cyanocobalamin.....	0.5mg
Folic acid.....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar add glucose and Na<sub>2</sub>S·9H<sub>2</sub>O. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

#### MSV I Agar

<b>Composition per liter:</b>	
Glucose.....	0.15g
MSV agar.....	1.0L
pH 7.2–7.5 at 25°C	

#### MSV Agar:

<b>Composition per liter:</b>	
Agar.....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
EDTA.....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O.....	2.0mg
Vitamin mix.....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Mix:

<b>Composition per 100.0mL:</b>	
Calcium pantothenate.....	0.01g
Niacin.....	0.01g
Pyridoxine.....	0.01g
<i>p</i> -Aminobenzoic acid.....	0.01g
Coccarboxylase.....	0.01g
Inositol.....	0.01g
Thiamine.....	0.01g
Riboflavin.....	0.01g
Biotin.....	0.5mg
Cyanocobalamin.....	0.5mg
Folic acid.....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar, add glucose. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

#### MSV LT Agar

##### Composition per liter:

Sodium lactate.....	0.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	0.5g
MSV agar.....	1.0L
pH 7.2–7.5 at 25°C	

#### MSV Agar:

##### Composition per liter:

Agar.....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
EDTA.....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O.....	2.0mg
Vitamin mix.....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Mix:

##### Composition per 100.0mL:

Calcium pantothenate.....	0.01g
Niacin.....	0.01g
Pyridoxine.....	0.01g
<i>p</i> -Aminobenzoic acid.....	0.01g
Coccarboxylase.....	0.01g
Inositol.....	0.01g
Thiamine.....	0.01g
Riboflavin.....	0.01g
Biotin.....	0.5mg
Cyanocobalamin.....	0.5mg
Folic acid.....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar, add sodium lactate and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

#### MSV S Agar

##### Composition per liter:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.187g
MSV agar.....	1.0L
pH 7.2–7.5 at 25°C	

#### MSV Agar:

##### Composition per liter:

Agar.....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
EDTA.....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O.....	2.0mg
Vitamin mix.....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin Mix:****Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g
Pyridoxine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	0.01g
Coccarboxylase .....	0.01g
Inositol .....	0.01g
Thiamine .....	0.01g
Riboflavin .....	0.01g
Biotin .....	0.5mg
Cyanocobalamin .....	0.5mg
Folic acid .....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar, add Na<sub>2</sub>S·9H<sub>2</sub>O. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

**MSV SS Agar****Composition per liter:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.187g
Sucrose .....	0.15g
MSV agar .....	1.0L

pH 7.2–7.5 at 25°C

**MSV Agar:****Composition per liter:**

Agar .....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
EDTA .....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	2.0mg
Vitamin mix .....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin Mix:****Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g
Pyridoxine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	0.01g
Coccarboxylase .....	0.01g
Inositol .....	0.01g
Thiamine .....	0.01g
Riboflavin .....	0.01g
Biotin .....	0.5mg
Cyanocobalamin .....	0.5mg
Folic acid .....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar, add Na<sub>2</sub>S·9H<sub>2</sub>O and sucrose. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15

min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

**MSV SUC Agar****Composition per liter:**

Sodium succinate .....	0.15g
MSV agar .....	1.0L

pH 7.2–7.5 at 25°C

**MSV Agar:****Composition per liter:**

Agar .....	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.11g
KH <sub>2</sub> PO <sub>4</sub> .....	0.085g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
EDTA .....	3.0mg
FeCl <sub>3</sub> ·H <sub>2</sub> O .....	2.0mg
Vitamin mix .....	1.0mL

**Preparation of MSV Agar:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin Mix:****Composition per 100.0mL:**

Calcium pantothenate .....	0.01g
Niacin .....	0.01g
Pyridoxine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	0.01g
Coccarboxylase .....	0.01g
Inositol .....	0.01g
Thiamine .....	0.01g
Riboflavin .....	0.01g
Biotin .....	0.5mg
Cyanocobalamin .....	0.5mg
Folic acid .....	0.5mg

**Preparation of Vitamin Mix:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** To 1.0L of MSV agar, add sodium succinate. Adjust pH to 7.2–7.5. Gently heat to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and enrichment of heterotrophic strains of *Thiothrix* species from water and environmental sources.

**MSVP Agar****Composition per 984.0mL:**

Agar, noble .....	15.0g
HEPES ( <i>N</i> -[2-Hydroxyethyl]piperazine- <i>N'</i> -2-ethanesulfonic acid) buffer .....	2.383g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.24g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.06g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.03g
KH <sub>2</sub> PO <sub>4</sub> .....	0.02g
FeSO <sub>4</sub> (10mM solution) .....	1.0mL
Sodium pyruvate solution .....	5.0mL
Vitamin solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Sodium Pyruvate Solution:****Composition per 50.0mL:**

Sodium pyruvate ..... 10.0g

**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl ..... 100.0mg  
*p*-Aminobenzoic acid ..... 50.0mg  
 D-(+)-Calcium pantothenate ..... 50.0mg  
 Nicotinic acid ..... 50.0mg  
 Riboflavin ..... 50.0mg  
 Thiamine-HCl ..... 50.0mg  
 Biotin ..... 20.0mg  
 Folic acid ..... 20.0mg  
 Vitamin B<sub>12</sub> ..... 1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except sodium pyruvate solution and vitamin solution, to distilled/deionized water and bring volume to 994.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 5.0mL of sterile sodium pyruvate solution and 1.0mL of sterile vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Leptothrix discophora*.

**MTP4 Medium****Composition per 1001.0mL:**

Solution A ..... 870.0mL  
 Solution C ..... 100.0mL  
 Solution D (Vitamin solution) ..... 10.0mL  
 Solution E ..... 10.0mL  
 Solution B (Trace elements solution SL-10) ..... 1.0mL  
 Methanol ..... 1.0mL  
 Methanethiol gas ..... 1–2.0mL

pH 7.1–7.4 at 25°C

**Solution A:****Composition per 870.0mL:**

NaCl ..... 21.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.1g  
 KCl ..... 0.5g  
 NH<sub>4</sub>Cl ..... 0.3g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
 Resazurin ..... 1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg

NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
 HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution.

Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:****Composition per 100.0mL:**NaHCO<sub>3</sub> ..... 5.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Solution D (Vitamin Solution):****Composition per liter:**

Pyridoxine-HCl ..... 10.0mg  
 Calcium DL-pantothenate ..... 5.0mg  
 Lipoic acid ..... 5.0mg  
 Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
 Riboflavin ..... 5.0mg  
 Thiamine-HCl ..... 5.0mg  
 Biotin ..... 2.0mg  
 Folic acid ..... 2.0mg  
 Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Solution D (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E:****Composition per 10.0mL:**Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution E:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine solution A with solution B, solution C, solution D, and solution E, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Prior to inoculation, aseptically and anaerobically add 1.0mL of filter-sterilized methanol and 1.0–2.0mL of methanethiol gas to each liter of medium. Addition of 10–20mg of sodium dithionite per liter (e.g., from a 5% solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth at the beginning.

**Use:** For the cultivation and maintenance of *Methanosaerina* species.

**MV Medium****Composition per 1001.0mL:**

Na<sub>2</sub>SO<sub>4</sub> ..... 2.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.0g  
 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 1.0g  
 NH<sub>4</sub>Cl ..... 1.0g  
 Yeast extract ..... 1.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.5g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
 Resazurin ..... 0.5mg  
 Wolfe's vitamin solution ..... 10.0mL  
 Sodium malate solution ..... 10.0mL

Sodium pyruvate solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.0–7.2 at 25°C	

**Sodium Malate Solution:****Composition per 100.0mL:**

Sodium malate .....	1.0g
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**Preparation of Sodium Malate Solution:** Add sodium malate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Sodium Pyruvate Solution:****Composition per 100.0mL:**

Sodium pyruvate .....	1.0g
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**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.75μg
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thorough-

ly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Wolfe's vitamin solution, sodium malate solution, sodium pyruvate solution, NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 7.0–7.2. Gently heat and bring to boiling. Cool while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Wolfe's vitamin solution, 10.0mL of sterile sodium malate solution, 10.0mL of sterile sodium pyruvate solution, 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL of sterile trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Desulfotomaculum orientis*.

**MWY Medium****(Wadowsky-Yee Medium, Modified)****Composition per liter:**

Agar .....	13.0g
Yeast extract .....	10.0g
Glycine .....	3.0g
ACES buffer (2-[(2-Amino-2-oxoethyl)-amino]-ethane sulfonic acid) .....	2.0g
Charcoal, activated .....	2.0g
α-Ketoglutarate .....	0.2g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> ·9H <sub>2</sub> O .....	0.05g
Bromocresol Purple .....	0.01g
Bromocresol Blue .....	0.01g
Antibiotic inhibitor .....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
pH 6.9 ± 0.2 at 25°C	

**Antibiotic Inhibitor:****Composition per 10.0mL:**

Anisomycin .....	0.16g
Cefamandole .....	4.0mg
Vancomycin .....	1.0mg
Polymyxin B .....	130,000U

**Preparation of Antibiotic Inhibitor:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**L-Cysteine·HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.08g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except L-cysteine and antibiotic inhibitor, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust medium to pH 6.9 with 1N KOH. Heat gently and bring to boiling for 1 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Add 10.0mL of the sterile L-cysteine·HCl·H<sub>2</sub>O solution and 10.0mL of the sterile antibiotic solution. Mix thoroughly. Pour into sterile Petri dishes with constant agitation to keep charcoal in suspension.

**Use:** For the selective isolation and cultivation of *Legionella pneumophila* and other *Legionella* species.

**MY Agar****Composition per liter:**

Agar .....	15.0g
Sodium acetate .....	0.1g
Yeast extract .....	0.1g
Pancreatic digest of gelatin .....	0.06g
Beef extract .....	0.04g
Basal medium .....	1.0L
Sodium sulfide solution .....	3.0mL
pH 7.0–7.5 at 25°C	

**Basal Medium:****Composition per liter:**

CaSO <sub>4</sub> ·2H <sub>2</sub> O (saturated solution) .....	20.0mL
NH <sub>4</sub> Cl (4% solution) .....	5.0mL
Trace elements solution .....	5.0mL
K <sub>2</sub> HPO <sub>4</sub> (1% solution) .....	1.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O (1% solution) .....	1.0mL

**Preparation of Basal Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:****Composition per liter:**

Ferrous EDTA solution .....	20.0mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.1% solution) .....	10.0mL
MnSO <sub>4</sub> ·4H <sub>2</sub> O (0.02% solution) .....	10.0mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.0005% solution) .....	10.0mL
H <sub>3</sub> BO <sub>3</sub> (0.1% solution) .....	10.0mL
Co(NO <sub>3</sub> ) <sub>2</sub> or	
CoCl <sub>2</sub> ·6H <sub>2</sub> O (0.01% solution) .....	10.0mL
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O (0.01% solution) .....	10.0mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Ferrous EDTA Solution:****Composition per 100.0mL:**

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
EDTA .....	2.0g
HCl, concentrated .....	1.0mL

**Preparation of Ferrous EDTA Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Sodium Sulfide Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Sodium Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Prepare freshly.

**Preparation of Medium:** Add sodium acetate, beef extract, pancreatic digest of gelatin, yeast extract, and agar to 1.0L of basal medium. Mix thoroughly. Adjust pH to 7.0–7.5. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 3.0mL of sterile sodium sulfide solution immediately prior to dispensing. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile screw-capped tubes.

**Use:** For the isolation and cultivation of *Beggiatoa* species and myxotrophic strains of *Thiothrix* species from water and environmental sources.

**MY20 Agar****Composition per liter:**

Glucose .....	200.0g
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Agar .....	20.0g
Peptone .....	5.0g
Yeast extract .....	3.0g
Malt extract .....	3.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of osmophilic heterotrophic bacteria and fungi.

**MY40 Agar****Composition per liter:**

Sucrose .....	400.0g
Agar .....	20.0g
Malt extract .....	20.0g
Yeast extract .....	5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of osmophilic heterotrophic bacteria and fungi.

**MY60 Agar****Composition per liter:**

Sucrose .....	600.0g
Agar .....	20.0g
Malt extract .....	20.0g
Yeast extract .....	5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of osmophilic heterotrophic bacteria and fungi.

**Mycobacterium Medium****Composition per liter:**

Noble agar .....	15.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
MnSO <sub>4</sub> .....	2.0mg
Liquid paraffin .....	5.0mL

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Homogenize in a blender. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Mycobacterium paraffinicum*.

**Mycobacterium Yeast Extract Medium****Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	2.5g
Glucose .....	1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Mycobacterium* species and *Rhodococcus* species.

### Mycorrhiza Medium

#### Composition per liter:

Agar .....	15.0g
Glucose .....	4.0g
Ammonium tartrate .....	1.0g
Malt extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	26.0mg
NaCl .....	20.0mg
Inositol .....	10.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.88mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.81mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.8mg
Nicotinamide .....	100.0µg
p-Aminobenzoic acid .....	100.0µg
Pantothenic acid .....	100.0µg
Pyridoxine .....	100.0µg
Thiamine .....	100.0µg
Biotin .....	25.0µg
Riboflavin .....	25.0µg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until boiling. Distribute into tubes or flasks. Autoclave for 10 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thelephora terrestris*.

### Myxobacteria Medium

#### Composition per liter:

Agar .....	15.0g
Skim milk powder .....	5.0g
Yeast extract .....	0.5g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Do not adjust pH. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Archangium primigenium*, *Chondrococcus macrosporus*, and *Myxococcus coralloides*.

### Myxococcus flavescens Medium

#### Composition per liter:

Agar .....	15.0g
Soluble starch .....	5.0g
Casitone .....	2.5g
Galactose .....	1.0g
Raffinose .....	1.0g
Sucrose .....	1.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g

pH 6.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Adjust pH to 6.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Myxococcus flavescens*.

### Myxococcus Medium

#### Composition per liter:

Agar .....	12.0g
Pancreatic digest of casein .....	1.0g
Meat extract .....	1.0g
Glucose solution .....	50.0mL

pH 7.2 ± 0.2 at 25°C

#### Glucose Solution:

##### Composition per 50.0mL:

Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes or bottles. Allow tubes or bottles to cool in a slanted position.

**Use:** For the cultivation of *Myxococcus* species.

### Myxococcus xanthus Medium

Agar .....	20.0g
Pancreatic digest of casein .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.148g
KH <sub>2</sub> PO <sub>4</sub> .....	0.017g

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Myxococcus xanthus*.

### Nannocystis Agar

#### Composition per liter:

Agar .....	15.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes. After the agar has solidified, overlay the surface with 0.5mL of a suspension of dead (autoclaved) *Escherichia coli* cells.

**Use:** For the cultivation and maintenance of *Nannocystis* species.

### Naphthalene Medium

#### Composition per liter:

NH <sub>4</sub> NO <sub>3</sub> .....	2.5g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	1.0g
Naphthalene .....	0.64g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Fe(SO <sub>4</sub> ) <sub>3</sub> ·5H <sub>2</sub> O .....	0.01g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	5.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	0.5mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.1mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas alcaligenes*.

#### **Naphthalene Sulfonic Acid Medium**

##### **Composition per 1004.0mL:**

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.31g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.05g
Solution A .....	100.0mL
Solution B .....	3.0mL
Trace elements solution SL-4 .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

##### **Solution A:**

##### **Composition per liter:**

Glucose .....	3.0g
Glycerol .....	3.0g
Sodium succinate .....	3.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

##### **Solution B:**

##### **Composition per liter:**

Naphthalene sulfonic acid .....	2.3g
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**Preparation of Solution B:** Add naphthalene sulfonic acid to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

##### **Trace Elements Solution SL-4:**

##### **Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

##### **Trace Elements Solution SL-6:**

##### **Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except solution A, solution B, and trace elements solution SL-4, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 200.0mL of sterile solution A,

3.0mL of sterile solution B, and 1.0mL of sterile trace elements solution SL-4. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Pseudomonas putida*.

#### **Natroniella Medium (DSMZ Medium 784)**

##### **Composition per liter:**

Na <sub>2</sub> CO <sub>3</sub> .....	68.3g
NaCl .....	15.7g
NH <sub>4</sub> Cl .....	1.0g
KCl .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Yeast extract .....	0.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	100.0mL
Vitamin solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Ethanol solution .....	10.0mL
Trace elements solution SL-4 .....	1.0mL
pH 9.7–10.0 at 25°C	

##### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

##### **Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

##### **NaHCO<sub>3</sub> Solution:**

##### **Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	38.3g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

##### **Ethanol Solution:**

##### **Composition per 10.0mL:**

Ethanol .....	5.0mL
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**Preparation of Ethanol Solution:** Add ethanol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

##### **Vitamin Solution:**

##### **Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

##### **Trace Elements Solution SL-4:**

##### **Composition per liter:**

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

# Trace Elements Solution SL-6:

## Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except vitamin solution, NaHCO<sub>3</sub> solution, ethanol, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL sterile vitamin solution, 100.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL sterile ethanol solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 9.7–10.0. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Natroniella acetigena* (*Acetohalobium* sp.).

# Natronincola histidinovorans Medium (DSMZ Medium 930)

## Composition per liter:

NaCl .....	80.0g
Na <sub>2</sub> CO <sub>3</sub> .....	6.83g
NaHCO <sub>3</sub> .....	3.83g
NH <sub>4</sub> Cl .....	1.0g
KCl .....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	0.01g
Histidine solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Yeast extract solution .....	10.0mL
Vitamin solution .....	2.0mL
Trace elements solution .....	1.0mL

pH 8.9 ± 0.2 at 25°C

## Histidine Solution:

### Composition per 50.0mL:

Histidine .....	5.0g
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**Preparation of Histidine Solution:** Add histidine to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

## Yeast Extract Solution:

### Composition per 10.0mL:

Yeast extract .....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

# Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

## Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

## Trace Elements Solution:

### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

## Vitamin Solution:

### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub>, NH<sub>4</sub>Cl, Na<sub>2</sub>CO<sub>3</sub>, histidine solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, yeast extract solution, and vitamin solution, to distilled/deionized water and bring volume to 928.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add solid NaHCO<sub>3</sub>, NH<sub>4</sub>Cl, and Na<sub>2</sub>CO<sub>3</sub>. Mix thoroughly. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium 50.0mL histidine solution, 10.0mL yeast extract solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 2.0mL vitamin solution. The final pH should be 8.9.

**Use:** For the cultivation of *Natronincola histidinovorans*.

# Natronobacteria Medium

## Composition per liter:

NaCl .....	200.0g
Agar .....	20.0g



Yeast extract.....	5.0g
Casamino acids.....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
KCl.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
Sodium glutamate.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.24g
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.17g
Na <sub>2</sub> CO <sub>3</sub> solution.....	100.0mL
Trace elements solution SL-6.....	1.0mL
pH 9.0 ± 0.2 at 25°C	

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	5.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Trace Elements Solution SL-6:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 9.0, if necessary. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Natronobacterium gregoryi*, *Natronobacterium magadii*, *Natronobacterium pharaonis*, and *Natronococcus occultus*.

***Natronobacterium pharaonis* Medium****Composition** per liter:

NaCl.....	250.0g
Casamino acids.....	15.0g
Sodium citrate.....	3.0g
Glutamic acid.....	2.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
KCl.....	2.0g
pH 8.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Natronobacterium pharaonis*.

***Nautilia* Medium  
(DSMZ Medium 946)****Composition** per liter:

Sulfur.....	10.0g
NH <sub>4</sub> Cl.....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
Resazurin.....	0.5mg
Synthetic seawater, concentrated.....	500.0mL

NaHCO <sub>3</sub> solution.....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	20.0mL
Sodium formate solution.....	15.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
Selenite-tungstate solution.....	1.0mL
pH 6.8 ± 0.2 at 25°C	

**Synthetic Seawater, Concentrated:**

NaCl.....	55.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	14.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.5g
KCl.....	1.3g
NaBr.....	0.2g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
SrCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.03g
Na <sub>3</sub> -citrate.....	20.0mg
KI.....	0.1mg

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitritotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Sodium Formate Solution:****Composition** per 50.0mL:

Na-formate.....	10.0g
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**Preparation of Sodium Formate Solution:** Add sodium formate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 20mL:**Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.6g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Selenite-Tungstate Solution****Composition per liter:**

NaOH ..... 0.5g

Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O ..... 4.0mgNa<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Sulfur:** Sterilize sulfur by steaming for 3 hr on each of 3 successive days.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except sulfur, NaHCO<sub>3</sub> solution, sodium formate solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, vitamin solution, selenite-tungstate solution, and trace elements solution, to distilled/deionized water and bring volume to 894.0mL. Mix thoroughly. Adjust pH to 6.8. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0g steam sterilized sulfur, 50.0mL NaHCO<sub>3</sub> solution, 15.0mL sodium formate solution, 20.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL vitamin solution, 1.0mL selenite-tungstate solution, and 10.0mL trace elements solution. Mix thoroughly. Adjust pH to 6.8. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Caldithrix abyssi* and *Nautilia lithotrophica*.

**NBA Medium****Composition per liter:**

Pancreatic digest of gelatin ..... 5.0g

Casamino acids ..... 5.0g

Beef extract ..... 3.0g

Yeast extract ..... 1.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Bdellovibrio* species.

**NBY Medium****(Nutrient Broth Yeast Extract Medium)****Composition per liter:**

Nutrient broth, dehydrated ..... 8.0g

Yeast extract ..... 2.0g

K<sub>2</sub>HPO<sub>4</sub> ..... 2.0gKH<sub>2</sub>PO<sub>4</sub> ..... 0.5g

Glucose solution ..... 50.0mL

MgSO<sub>4</sub>·7H<sub>2</sub>O (1M solution) ..... 1.0mL**Glucose Solution:****Composition per 50.0mL:**

D-Glucose ..... 5.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Curtobacterium flaccumfaciens* and *Pseudomonas syringae*.

**Nevskia Medium  
(DSMZ Medium 828)****Composition per liter:**

Na-lactate ..... 0.56g

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.05gCaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.014g

Potassium phosphate buffer, 1M, pH 7 ..... 27.5mL

Trace elements solution SL-9 ..... 0.5mL

Seven vitamin solution ..... 0.5mL

pH 7.1 ± 0.2 at 25°C

**Trace Elements Solution SL-9:****Composition per liter:**

Nitrilotriacetic acid ..... 12.8g

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 3.5gCoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mgMnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mgZnCl<sub>2</sub> ..... 70.0mgNa<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mgNiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mgH<sub>3</sub>BO<sub>3</sub> ..... 6.0mgCuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg

HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-9:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 6.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Seven Vitamin Solution:****Composition per liter:**

Pyridoxine hydrochloride ..... 300.0mg

Thiamine-HCl·2H<sub>2</sub>O ..... 200.0mg

Nicotinic acid ..... 200.0mg

Vitamin B<sub>12</sub> ..... 100.0mg

Calcium pantothenate ..... 100.0mg

*p*-Aminobenzoic acid ..... 80.0mg

D(+)-Biotin ..... 20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except seven vitamin solution, to distilled/deionized water and bring volume to 999.5mL. Mix thoroughly. Adjust pH to 7.1. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 0.5mL seven vitamin solution.

Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Nevskia ramosa*.

### Nine K Medium (9K Medium)

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	50.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
H <sub>2</sub> SO <sub>4</sub> 10N .....	1.0mL
pH 3.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus ferrooxidans*.

### Nitrate Agar

**Composition** per liter:

Agar .....	12.0g
Peptone .....	5.0g
Beef extract .....	3.0g
KNO <sub>3</sub> .....	1.0g
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Use:** For the differentiation of aerobic and facultative Gram-negative microorganisms based on their ability to reduce nitrate. Test for nitrates with sulfanilic acid and  $\alpha$ -naphthylamine reagents. Bacteria that reduce nitrate to nitrite turn the reagents red or pink.

### Nitrate Liquid Medium

**Composition** per liter:

Solution A .....	500.0mL
Solution B .....	250.0mL
Solution C .....	250.0mL

#### Solution A:

**Composition** per 500.0mL:

Mannitol .....	10.0g
KNO <sub>3</sub> .....	0.6g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	0.45g
Na <sub>2</sub> SO <sub>4</sub> .....	0.03g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution B:

**Composition** per 250.0mL:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### Solution C:

**Composition** per 250.0mL:

Calcium pantothenate .....	0.5mg
Thiamine-HCl .....	0.1mg
Biotin .....	0.5µg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 500.0mL of cooled, sterile solution A, 250.0mL of cooled, sterile solution B, and 250.0mL of sterile solution C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Rhizobium* species.

### Nitrate Methanol Medium

**Composition** per liter:

NaNO <sub>3</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	1.0mg
Riboflavin .....	0.2mg
Calcium pantothenate .....	0.2mg
Pyridoxine-HCl .....	0.2mg
Nicotinic acid .....	0.2mg
Thiamine-HCl .....	0.1mg
p-Aminobenzoic acid .....	0.1mg
Biotin .....	0.01mg
Methanol .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Filter sterilize methanol. Aseptically add sterile methanol to cooled sterile medium. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Methylobacterium rhodinum*.

### Nitrate Mineral Salts Medium (NMS Medium)

**Composition** per liter:

Noble agar .....	12.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
KNO <sub>3</sub> .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	0.717g
KH <sub>2</sub> PO <sub>4</sub> .....	0.272g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Ferric ammonium EDTA .....	4.0mg
Trace elements solution .....	0.5mL
pH 6.8 ± 0.2 at 25°C	

### Trace Elements Solution:

**Composition** per liter:

Disodium EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
H <sub>3</sub> BO <sub>3</sub> .....	0.03g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	3.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Methylobacterium* species, *Methylococcus capsulatus*, *Methylomonas agilis*, and *Methylomonas methanica*.

#### Nitrate Mineral Salts Medium with Methanol (NMS Medium with Methanol)

**Composition per liter:**

Noble agar .....	12.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
KNO <sub>3</sub> .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	0.717g
KH <sub>2</sub> PO <sub>4</sub> .....	0.272g
CaCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Ferric ammonium EDTA .....	4.0mg
Trace elements solution .....	0.5mL
Methanol .....	1.0mL

pH 6.8 ± 0.2 at 25°C

#### Trace Elements Solution:

**Composition per liter:**

Disodium EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
H <sub>3</sub> BO <sub>3</sub> .....	0.03g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	3.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	3.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Filter sterilize methanol. Aseptically add sterile methanol to cooled sterile medium. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Methylobacterium fujisawaense*, *Methylobacterium* species, and *Methylomonas clara*.

#### Nitrate Reduction Broth

**Composition per liter:**

Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
KNO <sub>3</sub> .....	1.0g

pH 6.9 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into test tubes that contain an inverted Durham tube. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the differentiation of members of the Pseudomonadaceae based on their ability to reduce nitrate

to nitrite or form N<sub>2</sub> gas. Test for nitrates with sulfanilic acid and α-naphthylamine reagents. Bacteria that reduce nitrate to nitrite turn the reagents red or pink.

#### Nitrate Reduction Broth for *Pseudomonas* and Related Genera

**Composition per liter:**

Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g
NaNO <sub>3</sub> .....	0.1g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into test tubes that contain an inverted Durham tube. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the differentiation of members of the Pseudomonadaceae based on their ability to reduce nitrate to nitrite or form N<sub>2</sub> gas. Test for nitrates with sulfanilic acid and α-naphthylamine reagents. Bacteria that reduce nitrate to nitrite turn the reagents red or pink.

#### *Nitrobacter agilis* Medium

**Composition per liter:**

CaCO <sub>3</sub> .....	10.0g
NaCl .....	0.3g
Na <sub>2</sub> CO <sub>3</sub> .....	0.25g
KNO <sub>2</sub> .....	0.17g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.14g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.03g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
Biotin solution .....	10.0mL

#### Biotin Solution:

**Composition per 10.0mL:**

Biotin .....	0.15g
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**Preparation of Biotin Solution:** Add biotin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. In a separate flask, add the remaining components, except the biotin solution, to distilled/deionized water and bring volume to 790.0mL. Autoclave the Na<sub>2</sub>CO<sub>3</sub> solution and salts solution separately for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically combine the sterile Na<sub>2</sub>CO<sub>3</sub> solution, sterile salts solution, and sterile biotin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Nitrobacter agilis*.

#### *Nitrobacter* Medium 203

**Composition per liter:**

Solution C .....	1.0mL
Solution A .....	0.5mL
Solution B .....	0.5mL
Solution D .....	0.5mL
Solution E .....	0.5mL
Solution F .....	0.2mL

#### Solution A:

**Composition per 100.0mL:**

CaCl <sub>2</sub> .....	2.0g
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**Preparation of Solution A:** Add  $\text{CaCl}_2$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution B:**

**Composition** per 100.0mL:

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 20.0g

**Preparation of Solution B:** Add  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution C:**

**Composition** per 100.0mL:

Chelated iron (Sequestrene) ..... 0.1g

**Preparation of Solution C:** Add chelated iron to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution D:**

**Composition** per liter:

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 0.2g

$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 0.1g

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 0.02g

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 2.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Solution E:**

**Composition** per 100.0mL:

$\text{NaNO}_2$  ..... 41.4g

**Preparation of Solution E:** Add  $\text{NaNO}_2$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution F:**

**Composition** per 100.0mL:

$\text{K}_2\text{HPO}_4$  ..... 1.74g

**Preparation of Solution F:** Add  $\text{K}_2\text{HPO}_4$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add the appropriate volumes of solutions A–F to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Nitrobacter* species and *Nitrobacter winogradskyi*.

### *Nitrobacter Medium 204*

**Composition** per liter:

Seawater ..... 700.0mL

Solution C ..... 1.0mL

Solution A ..... 0.5mL

Solution B ..... 0.5mL

Solution D ..... 0.5mL

Solution E ..... 0.5mL

Solution F ..... 0.2mL

**Solution A:**

**Composition** per 100.0mL:

$\text{CaCl}_2$  ..... 2.0g

**Preparation of Solution A:** Add  $\text{CaCl}_2$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution B:**

**Composition** per 100.0mL:

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 20.0g

**Preparation of Solution B:** Add  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution C:**

**Composition** per 100.0mL:

Chelated iron (Sequestrene) ..... 0.1g

**Preparation of Solution C:** Add chelated iron to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution D:**

**Composition** per liter:

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 0.2g

$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 0.1g

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 0.02g

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 2.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Solution E:**

**Composition** per 100.0mL:

$\text{NaNO}_2$  ..... 41.4g

**Preparation of Solution E:** Add  $\text{NaNO}_2$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution F:**

**Composition** per 100.0mL:

$\text{K}_2\text{HPO}_4$  ..... 1.74g

**Preparation of Solution F:** Add  $\text{K}_2\text{HPO}_4$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add the appropriate volumes of solutions A–F and seawater to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Nitrococcus mobilis*.

### *Nitrobacter Medium B*

**Composition** per liter:

$\text{NaNO}_2$  ..... 1.0g

$\text{K}_2\text{HPO}_4$  ..... 0.5g

$\text{MgSO}_4$  ..... 0.5g

$\text{NaCl}$  ..... 0.3g

$\text{Fe}_2(\text{SO}_4)_3$  ..... 5.0mg

$\text{MnSO}_4$  ..... 2.0mg

Marble chips ..... as needed

pH  $7.5 \pm 0.2$  at 25°C

**Preparation of Medium:** Add components, except marble chips, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Wash marble chips in distilled/deionized water. Put a few chips into test tubes. Autoclave for 60 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically distribute cooled sterile medium into test tubes to cover marble chips.

**Use:** For the cultivation of *Nitrobacter* species.

**Nitrococcus Medium****Composition** per 1004.0mL:

NaNO <sub>2</sub> solution .....	1.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	1.0mL
NaHCO <sub>3</sub> solution .....	1.0mL
Chelated metals solution .....	1.0mL
pH 7.5 ± 0.1 at 25°C	

**NaNO<sub>2</sub> Solution:****Composition** per 100.0mL:

NaNO <sub>2</sub> solution .....	10.0g
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**Preparation of NaNO<sub>2</sub> Solution:** Add NaNO<sub>2</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**K<sub>2</sub>HPO<sub>4</sub> Solution:****Composition** per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> solution .....	2.5g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**NaHCO<sub>3</sub> Solution:****Composition** per 100.0mL:

NaHCO <sub>3</sub> solution .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Chelated Metals Solution:****Composition** per liter:

EDTA .....	6.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.6g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.15g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	4.0mg

**Preparation of Chelated Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Adjust pH of 1.0L of seawater to pH 7.5 with NaOH. Add 1mL of chelated metals solution to the seawater. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 1.0mL each of sterile NaNO<sub>2</sub>, K<sub>2</sub>HPO<sub>4</sub>, and NaHCO<sub>3</sub> solutions. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Nitrococcus* species.

**Nitrogen-Fixing Hydrocarbon Oxidizers Medium****Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and enrichment of nitrogen-fixing hydrocarbon-oxidizing bacteria.

**Nitrogen-Fixing Marine Medium****Composition** per liter:

Noble agar .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
Citric acid .....	3.0mg
Ferric ammonium citrate .....	3.0mg
Disodium potassium EDTA .....	0.5mg
Seawater .....	750.0mL
Trace metals A-5 .....	1.0mL
pH 8.5 ± 0.2 at 25°C	

**Trace Metals A-5 Mix:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.05g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.039g

**Preparation of Trace Metals A-5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to glass-distilled water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 8.5 with KOH. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Anabaena* species.

**Nitrogen-Free Agar****Composition** per liter:

Agar .....	15.0g
CaCO <sub>3</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	5.0mg
Glucose solution .....	50.0mL
pH 7.0 ± 0.2 at 25°C	

**Glucose Solution:****Composition** per 50.0mL:

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and agar, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Add agar. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Azomonas agilis*, *Azomonas insignis*, *Azomonas macrocytogenes*, *Azorhizophilus paspali*, *Azotobacter beijerinckii*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Beijerinckia acida*, *Beijerinckia fluminensis*, *Beijerinckia indica*, *Beijerinckia mobilis*, and *Derxia gummosa*.

### Nitrogen-Free Agar (Norris Agar)

#### Composition per liter:

Agar .....	15.0g
CaCO <sub>3</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	5.0mg
Glucose solution .....	50.0mL

#### Glucose Solution:

##### Composition per 100.0mL:

Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add 20.0g of glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 50°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Azomonas agilis*, *Azotobacter chroococcum*, and *Azotobacter vinelandii*.

### Nitrosococcus Medium

#### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.32g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.38g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
K <sub>2</sub> HPO <sub>4</sub> .....	8.7mg
Chelated iron .....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0µg
Phenol Red (0.04% solution) .....	3.25mL

pH 7.5–7.8 at 25°C

**Preparation of Medium:** Add components to filtered seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5–7.8 with 1N HCl. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Nitrosococcus oceanus*.

### Nitrosococcus oceanus Medium

#### Composition per 1001.0mL:

Phenol red .....	5.0g
NH <sub>4</sub> Cl .....	0.635g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.357g
K <sub>2</sub> HPO <sub>4</sub> .....	43.0mg
CaCl <sub>2</sub> ·H <sub>2</sub> O .....	20.0mg
Chelated metals solution .....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Chelated Metals Solution:

##### Composition per liter:

EDTA .....	6.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.6g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.15g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	4.0mg

**Preparation of Chelated Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except chelated metals solution, to filtered seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with sterile 0.1M K<sub>2</sub>CO<sub>3</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mL of sterile chelated metals solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Nitrosococcus oceanus*.

### Nitrosolobus Medium (ATCC Medium 438)

#### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.65g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.087g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
Phenol Red .....	5.0mg
Disodium EDTA .....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.02mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0µg

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with 0.1M K<sub>2</sub>CO<sub>3</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Nitrosolobus multiformis*.

### Nitrosolobus Medium (ATCC Medium 929)

#### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.32g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.38g
K <sub>2</sub> HPO <sub>4</sub> .....	0.087g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
Chelated iron .....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.0µg
Phenol Red (0.5% solution) .....	0.25mL

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with 0.1M K<sub>2</sub>CO<sub>3</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Nitrosolobus multiformis*.

### Nitrosomonas europaea Medium

#### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
K <sub>2</sub> HPO <sub>4</sub> .....	0.015g
Ferric EDTA .....	1.0mg
Trace elements solution .....	1.0mL

pH 7.5 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per 100.0mL:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	2.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5 with K<sub>2</sub>CO<sub>3</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. After inoculation, maintain pH at 7.5–7.8 with sterile 50% K<sub>2</sub>CO<sub>3</sub> solution.

**Use:** For the cultivation and maintenance of *Nitrosomonas europaea*.

***Nitrosomonas* Medium****Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	4.0mg
Cresol Red (0.0005% solution) .....	25.0mL
Ferric EDTA solution .....	0.1mL

pH 8.2–8.4 at 25°C

**Ferric EDTA Solution:****Composition** per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Disodium EDTA .....	0.14g
H <sub>2</sub> SO <sub>4</sub> , concentrated .....	0.05mL

**Preparation of Ferric EDTA Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O and MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. In a separate flask, add remaining components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave both solutions separately for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically combine the two sterile solutions. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. After inoculation, maintain pH at 8.2–8.4 with sterile 50% K<sub>2</sub>CO<sub>3</sub> solution.

**Use:** For the cultivation and maintenance of *Nitrosomonas europaea*.

***Nitrospira moscoviensis* Medium  
(DSMZ Medium 756d)****Composition** per liter:

NaNO <sub>2</sub> .....	0.5g
Stock solution .....	100.0mL
Trace elements solution .....	1.0mL

pH 8.6 ± 0.2 at 25°C

**Stock Solution:****Composition** per liter:

NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCO <sub>3</sub> .....	0.07g

**Preparation of Stock Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:****Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	97.3mg
H <sub>3</sub> BO <sub>3</sub> .....	49.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	43.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	37.1mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	33.8mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	25.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.6. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Allow to stand for 2–3 days so that pH adjusts itself to 7.4–7.6.

**Use:** For the cultivation of *Nitrospira moscoviensis*.

**NSMP, Modified****Composition** per liter:

Casamino acids .....	5.0g
Glucose .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.86g
Sodium citrate .....	0.6g
K <sub>2</sub> HPO <sub>4</sub> .....	0.55g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.43g
CaCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.016g
ZnCl <sub>2</sub> .....	7.0mg
FeCl <sub>3</sub> .....	3.0mg

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Bacillus thuringiensis*.

**Nutrient Agar  
(LMG Medium 160)****Composition** per liter:

Agar .....	3.0g
Lab-Lemco beef extract .....	1.0g
Peptone .....	1.0g
NaCl .....	0.5g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of heterotrophic bacteria.

**Nutrient Agar****Composition** per liter:

Agar .....	15.0g
Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g

pH 7.4 ± 0.2 at 25°C



**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a wide variety of microorganisms.

#### **Nutrient Agar (ATCC Medium 3)**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a wide variety of bacteria and for the enumeration of organisms in water, sewage, feces, and other materials.

#### **Nutrient Agar pH 6.8 (LMG Medium 2)**

**Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
NaCl .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> · 12 H <sub>2</sub> O .....	2.39g
Yeast extract .....	2.0g
Lab-Lemco beef extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.45g
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Acidovorax facilis*, *Pseudomonas geniculata*, *Arthrobacter siderocapsulatus*, *Delftia acidovorans*, *Pseudomonas* spp., and various other heterotrophic bacteria.

#### **Nutrient Agar pH 9.0 (ATCC Medium 209)**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 9.0 ± 0.1 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 9.0. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of alkilophilic bacteria.

#### **Nutrient Agar pH 10.0 (ATCC Medium 2030)**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 10.0 ± 0.1 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 10.0. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of alkilophilic bacteria.

#### **Nutrient Agar with 1% Methanol (ATCC Medium 620)**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
Methanol .....	10.0mL
pH 6.8 ± 0.2 at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Filter sterilize methanol. Add components, except methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bacillus* species, *Methylobacterium clara*, and *Pseudomonas methanolic*.

#### **Nutrient Agar with 2% Methanol (ATCC Medium 628)**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
Methanol .....	20.0mL
pH 6.8 ± 0.2 at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Filter sterilize methanol. Add components, except methanol, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile methanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

#### **Nutrient Agar with 0.5% NaCl**

**Composition per liter:**

Agar .....	15.0g
NaCl .....	5.0g

Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.8 ± 0.2 at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Agrobacterium tumefaciens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Shigella dysenteriae*, *Shigella flexneri*, *Vibrio* species, and *Yersinia* species.

#### Nutrient Agar with 0.5% NaCl, pH 9.5–10.0 (LMG Medium 253)

**Composition per liter:**

Agar .....	15.0g
NaCl .....	10.0g
Peptone .....	5.0g
Yeast extract .....	2.0g
Lab-Lemco beef extract .....	1.0g
pH 9.5–10.0	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Adjust pH to 9.5–10.0 with sterile Na<sub>2</sub>CO<sub>3</sub> solution. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus* spp.

#### Nutrient Agar with 1.5% NaCl

**Composition per liter:**

NaCl .....	15.0g
Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.8 ± 0.2 at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Photobacterium leiognathi*, *Pseudomonas fluorescens*, and *Vibrio natriegens*.

#### Nutrient Agar with 2% NaCl

**Composition per liter:**

NaCl .....	20.0g
Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Vibrio alginolyticus*, *Vibrio bivalvii*, *Vibrio mediterranei*, *Vibrio natriegens*, *Vibrio ordali*, *Vibrio orientalis*, *Vibrio parahaemolyticus*, *Vibrio pelagius*, and *Vibrio vulnificus*.

#### Nutrient Agar with 3% NaCl

**Composition per liter:**

NaCl .....	30.0g
Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.8 ± 0.2 at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus* species, *Alteromonas nigrifaciens*, *Halococcus* species, *Planococcus citreus*, *Pseudomonas beijerinckii*, *Staphylococcus* species, *Streptococcus pyogenes*, and *Vibrio* species.

#### Nutrient Agar with 5% NaCl, pH 9.5–10.0 (LMG Medium 254)

**Composition per liter:**

NaCl .....	55.0g
Agar .....	15.0g
Peptone .....	5.0g
Yeast extract .....	2.0g
Lab-Lemco beef extract .....	1.0g
pH 9.5–10.0	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Adjust pH to 9.5–10.0 with sterile Na<sub>2</sub>CO<sub>3</sub> solution. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus halalkaliphilus*.

#### Nutrient Agar with 10% NaCl

**Composition per liter:**

NaCl .....	100.0g
Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH 6.8 ± 0.2 at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Paracoccus halodenitrificans* and *Micrococcus* species.

#### Nutrient Agar with 10% NaCl and Maltose

**Composition per liter:**

NaCl .....	100.0g
Agar .....	15.0g
Maltose .....	10.0g

Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH $6.8 \pm 0.2$ at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Paracoccus halodenitrificans* and *Micrococcus* species.

#### Nutrient Agar with Phytone

**Composition per liter:**

Agar .....	15.0g
Phytone .....	10.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH $6.8 \pm 0.2$ at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a wide variety of bacteria.

#### Nutrient Agar with Soil Extract

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
Soil extract .....	250.0mL
pH $6.8 \pm 0.2$ at 25°C	

**Source:** Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

#### Soil Extract:

**Composition per 300.0mL:**

African Violet soil .....	115.5g
Na <sub>2</sub> CO <sub>3</sub> .....	0.3g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 300.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and maintenance of *Aerobacterium* species, *Bacillus* species, and *Saccharomonospora viridis*.

#### Nutrient Agar with 2% Sucrose (ATCC Medium 1297)

**Composition per liter:**

Sucrose .....	20.0 g
Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH $6.8 \pm 0.2$ at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of osmophilic bacteria

#### Nutrient Broth

**Composition per liter:**

Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g
pH $7.4 \pm 0.2$ at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of a wide variety of nonfastidious microorganisms.

#### Nutrient Broth

**Composition per liter:**

Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH $6.9 \pm 0.2$ at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of a wide variety of nonfastidious microorganisms.

#### Nutrient Broth with 6% NaCl

**Composition per liter:**

NaCl .....	60.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g
pH $6.8 \pm 0.2$ at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of organisms in water, sewage, feces, and other materials. For the cultivation and maintenance of *Paracoccus halodenitrificans*.

#### Nutrient Broth with Rifampicin

**Composition per liter:**

Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g
Rifampicin solution .....	10.0mL
pH $7.4 \pm 0.2$ at 25°C	

#### Rifampicin Solution:

**Composition per 10.0mL:**

Rifampicin .....	10.0mg
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**Preparation of Rifampicin Solution:** Add rifampicin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except rifampicin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL of sterile rifampicin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Agrobacterium tumefaciens*.

#### Nutrient Gelatin

**Composition per liter:**

Gelatin.....	120.0g
Pancreatic digest of gelatin.....	5.0g
Beef extract.....	3.0g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring to 50°C. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of bacteria based on their ability to liquefy gelatin.

#### Nutrient Soil Extract Agar (LMG Medium 30)

**Composition per liter:**

Agar.....	15.0g
Yeast extract.....	2.0g
Peptone.....	5.0g
NaCl.....	5.0g
Lab-Lemco beef extract.....	1.0g
Soil extract.....	1.0L

pH 7.4 ± 0.2 at 25°C

**Soil Extract:**

**Composition per liter:**

Soil.....	1.0kg
CaCO <sub>3</sub> .....	2.0g

**Preparation of Soil Extract:** Add 1.0kg soil to 1.0L tap water. Autoclave for 30 min at 15 psi pressure–121°C. Add 2.0g CaCO<sub>3</sub>. Filter through Whatman filter paper. Bring volume to 1.0L with tap water. Mix thoroughly.

**Preparation of Medium:** Add components to 1.0L soil extract. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of soil bacteria.

#### Nutrient Yeast Glucose Medium

**Composition per liter:**

Glucose.....	10.0g
Pancreatic digest of gelatin.....	5.0g
Yeast extract.....	5.0g
Beef extract.....	3.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Erwinia amylovora*.

#### NWRI Agar (HPC Agar)

**Composition per liter:**

Agar.....	15.0g
Peptone.....	3.0g
Soluble casein.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> .....	0.05g
FeCl <sub>3</sub> .....	1.0mg

pH 7.2 ± 0.2 at 25°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For estimation of the number of live heterotrophic bacteria in water using the heterotrophic plate count technique.

#### Oat Flake Medium (DSMZ Medium 189)

**Composition per liter:**

Oat flakes.....	30.0g
Agar.....	15.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add rolled oats to 500.0mL distilled/deionized water. Gently heat and bring to boiling. Boil for 10 min. Filter. Add agar and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Mix by shaking. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C. Mix by shaking. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of actinomycetes and fungi.

#### *Oceanithermus profundus* Medium (DSMZ Medium 975)

**Composition per liter:**

NaCl.....	30.0g
HEPES.....	2.38g
NH <sub>4</sub> Cl.....	0.33g
KCl.....	0.33g
Potassium nitrate solution.....	10.0mL
Calcium chloride solution.....	10.0mL
Magnesium chloride solution.....	10.0mL
Yeast extract solution.....	10.0mL
Sucrose solution.....	10.0mL
Tryptone solution.....	10.0mL
Vitamin solution.....	1.0mL
Trace elements solution.....	1.0mL

pH 7.0–7.5 at 25°C

**Potassium Nitrate Solution:**

**Composition per 10.0mL:**

KNO <sub>3</sub> .....	0.33g
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**Preparation of Potassium Nitrate Solution:** Add KNO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Magnesium Chloride Solution:**

**Composition per 10.0mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.33g
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**Preparation of Magnesium Chloride Solution:**

Add MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring

volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Calcium Chloride Solution:**

##### **Composition per 10.0mL:**

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.33g

**Preparation of Calcium Chloride Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Yeast Extract Solution:**

##### **Composition per 10.0mL:**

Yeast extract ..... 0.2g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Sucrose Solution:**

##### **Composition per 10.0mL:**

Sucrose ..... 2.0g

**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Tryptone Solution:**

##### **Composition per 10.0mL:**

Tryptone ..... 1.0g

**Preparation of Tryptone Solution:** Add tryptone to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Trace Elements Solution:**

##### **Composition per liter:**

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitrilotriacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O ..... 0.01g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Vitamin Solution:**

##### **Composition per liter:**

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg

Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas atmosphere. Add components, except yeast extract solution, sucrose solution, calcium chloride solution, magnesium chloride solution, potassium nitrate solution, tryptone solution, vitamin solution, and trace elements solution, to distilled/deionized water and bring volume to 938.0mL. Mix thoroughly. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter, 10.0mL sucrose solution, 10.0mL yeast extract solution, 10.0mL potassium nitrate solution, 10.0mL tryptone solution, 10.0mL magnesium chloride solution, 10.0mL calcium chloride solution, 1.0mL vitamin solution, and 1.0mL trace elements solution. Mix thoroughly. Adjust pH to 7.0–7.5

**Use:** For the cultivation of *Oceanithermus profundus*.

### **Oil Agar Medium**

#### **Composition per liter:**

Agar, purified ..... 20.0g  
NaCl ..... 10.0g  
Oil powder ..... 10.0g  
NH<sub>4</sub>NO<sub>3</sub> ..... 1.0g  
MgSO<sub>4</sub> ..... 0.5g  
Amphotericin B solution ..... 10.0mL  
K<sub>2</sub>HPO<sub>4</sub> solution ..... 7.0mL  
KH<sub>2</sub>PO<sub>4</sub> solution ..... 3.0mL  
FeCl<sub>3</sub> ..... 0.1mL

#### **Oil Powder:**

##### **Composition per 10.0g:**

Hydrocarbon ..... 10.0g  
Silica gel ..... 10.0g  
Diethyl ether ..... 30.0mL

**Preparation of Oil Powder:** Add 10.0g of hydrocarbon to 30.0mL of diethyl ether. Mix thoroughly. Add 10.0g of silica gel. Allow ether to evaporate.

#### **Amphotericin B Solution:**

##### **Composition per 10.0mL:**

Amphotericin B ..... 0.01g

**Preparation of Amphotericin B Solution:** Add amphotericin B to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **K<sub>2</sub>HPO<sub>4</sub> Solution:**

##### **Composition per 100.0mL:**

K<sub>2</sub>HPO<sub>4</sub> ..... 10.0g

**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### **KH<sub>2</sub>PO<sub>4</sub> Solution:**

##### **Composition per 100.0mL:**

KH<sub>2</sub>PO<sub>4</sub> ..... 10.0g

**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components—except amphotericin B solution,  $K_2HPO_4$  solution, and  $KH_2PO_4$  solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile amphotericin B solution, 7.0mL of sterile  $K_2HPO_4$  solution, and 3.0mL of sterile  $KH_2PO_4$  solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and enumeration of hydrocarbon-utilizing bacteria by direct plating of estuarine water and sediment samples.

#### OSrt Broth (ATCC Medium 2340)

**Composition per liter:**

Yeast extract .....	2.0 g
Glycerol .....	10.0 mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into flasks or tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Geodermatophilus obscurus* subspecies *utahensis*.

#### Oxidation-Fermentation Medium (OF Medium)

**Composition per liter:**

NaCl .....	5.0g
Agar .....	2.5g
Pancreatic digest of casein .....	2.0g
$K_2HPO_4$ .....	0.3g
Bromothymol Blue .....	0.03g
Carbohydrate solution .....	100.0mL
pH 6.8 ± 0.1 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Carbohydrate Solution:**

**Composition per 100.0mL:**

Carbohydrate .....	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile carbohydrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For differentiating Gram-negative bacteria based upon determining the oxidative and fermentative metabolism of carbohydrates.

#### Oxidation-Fermentation Medium, Hugh-Leifson's (Hugh-Leifson's Oxidation Fermentation Medium)

**Composition per liter:**

NaCl .....	5.0g
Agar .....	3.0g
Peptone .....	2.0g
$K_2HPO_4$ .....	0.3g

Carbohydrate solution .....	100.0mL
Bromothymol Blue solution (0.2% ) .....	15.0mL
pH 7.1 ± 0.2 at 25°C	

**Carbohydrate Solution:**

**Composition per 100.0mL:**

Carbohydrate .....	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile carbohydrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For differentiating Gram-negative bacteria, such as *Vibrio* species, based upon determining the oxidative and fermentative metabolism of carbohydrates. Bacteria that ferment the carbohydrate turn the medium yellow.

#### Oxidation-Fermentation Medium, King's (King's OF Medium)

**Composition per liter:**

Base .....	900.0mL
Carbohydrate solution .....	100.0mL

**Base:**

**Composition per liter:**

Agar .....	3.0g
Pancreatic digest of casein .....	2.0g
Carbohydrate solution .....	100.0mL
Phenol Red (1.5% solution) .....	2.0mL
pH to 7.3 ± 0.2	

**Carbohydrate Solution:**

**Composition per 100.0mL:**

Carbohydrate .....	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile carbohydrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For differentiating bacteria based upon determining the oxidative and fermentative metabolism of carbohydrates. Bacteria that ferment the carbohydrate turn the medium yellow.

#### OZR Medium

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g

**Preparation of Medium:** Add components to seawater and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Leucothrix mucor*.

**PA-C Agar  
(mPA-C Agar)**

**Composition per liter:**

Agar .....	12.0g
L-Lysine-HCl .....	5.0g
NaCl .....	5.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	5.0g
Yeast extract .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g
Lactose .....	1.25g
Sucrose .....	1.25g
Xylose .....	1.25g
Ferric ammonium citrate .....	0.8g
Phenol Red .....	0.08g
Nalidixic acid .....	0.037g
Kanamycin .....	8.0mg

pH 7.2 ± 0.1 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective recovery and enumeration of *Pseudomonas aeruginosa* from water samples.

***Paracoccus alcaliphilus* Medium  
(DSMZ Medium 772)**

**Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	3.0g
Yeast extract .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Fe-citrate .....	30.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
CuSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5mg
Methanol .....	10.0mL
NaHCO <sub>3</sub> solution .....	variable

pH 9.0 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:**

**Composition per 50.0mL:**

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution and methanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Filter sterilize 10.0mL of methanol. Aseptically add the 10.0mL filter sterilized methanol. Mix thoroughly. Adjust pH to 9.0 using the sterile NaHCO<sub>3</sub> solution.

**Use:** For the cultivation of *Paracoccus alcaliphilus*.

***Paracoccus aminophilus*  
*Paracoccus aminovorans* Medium  
(DSMZ Medium 774)**

**Composition per liter:**

Agar .....	20.0g
Peptone .....	5.0g
Yeast extract .....	5.0g
Glucose .....	5.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Paracoccus aminovorans* and *Paracoccus aminophilus*.

***Paracoccus halodenitrificans* Agar**

**Composition per liter:**

NaCl .....	60.0g
Agar .....	15.0g
Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	4.0g
Beef extract .....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Deleya aquamarina*, *Deleya halophila*, *Deleya venusta*, *Halomonas halophila*, *Marinococcus communis*, *Micrococcus halobius*, *Micrococcus varians*, and *Paracoccus halodenitrificans*.

***Paracoccus kocurii* Medium  
(DSMZ Medium 773)**

**Composition per liter:**

NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O .....	1.71g
K <sub>2</sub> HPO <sub>4</sub> .....	1.41g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Hutner's salts solution .....	20.0mL
Tetramethyl ammonium chloride solution .....	10.0mL
Thiamine hydrochloride solution .....	10.0mL

pH 6.9 ± 0.2 at 25°C

**Tetramethyl Ammonium Chloride Solution:**

**Composition per 10.0mL:**

Tetramethyl ammonium chloride .....	1.0g
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**Preparation of Tetramethyl Ammonium Chloride Solution:** Add tetramethyl ammonium chloride to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Thiamine Hydrochloride Solution:**

**Composition per 10.0mL:**

Thiamine-HCl·2H <sub>2</sub> O .....	0.5mg
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**Preparation of Thiamine Hydrochloride Solution:** Add thiamine-HCl·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Hutner's Salts Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitritotriacetic acid .....	10.0g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44" .....	50.0mL

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Salts Solution:** Add nitrotri-acetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Add components, except tetramethyl ammonium chloride solution and thiamine hydrochloride solution, to distilled/deionized water and bring volume to 980.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL sterile tetramethyl ammonium chloride solution and 10.0mL sterile thiamine hydrochloride solution. Mix thoroughly. Adjust pH to 6.9.

**Use:** For the cultivation of *Paracoccus kocurii*.

**Paraffin Agar****Composition per liter:**

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	6.0g
NH <sub>4</sub> NO <sub>3</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
Paraffin, liquid .....	1.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.049g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.046g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.4mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	2.5mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.94mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.2mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation and cultivation of streptomycetes.

**Paramecium Medium****Composition per liter:**

Solution C .....	500.0mL
Solution A .....	10.0mL
Solution B .....	1.0mL

**Solution A:****Composition per liter:**

Thiamine-HCl .....	1.5g
Calcium pantothenate .....	1.0g
Nicotinamide .....	0.5g
Pyridoxal-HCl .....	0.5g

Riboflavin .....	0.5g
Folic acid .....	0.5g
α-Lipoic acid .....	0.1g
Biotin .....	0.1mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute while stirring into screw-capped tubes in 10.0mL volumes. Store at –20°C. Thaw as needed.

**Solution B:****Composition per 100.0mL:**

TEM-4T (Hachmeister, Pittsburgh) .....	10.0g
Stigmasterol .....	0.5g
Ethanol, absolute .....	100.0mL

**Preparation of Solution B:** Add TEM-4T and stigmasterol to 100.0mL of hot ethanol. Mix thoroughly. Store at 4°C.

**Solution C:****Composition per 500.0mL:**

Protease peptone .....	10.0g
Pancreatic digest of casein .....	5.0g
Ribonucleic acid .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Preparation of Medium:** Combine 500.0mL of solution C, 10.0mL of solution A, and 1.0mL of solution B. Mix thoroughly. Bring volume to 1.0L with distilled/deionized water. Adjust pH to 7.0–7.2 with 0.1N NaOH. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Paramecium* species to be used as host cells by bacterial symbionts.

**Payne, Seghal, and Gibbons Medium****Composition per liter:**

NaCl .....	250.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	20.0g
Yeast extract .....	10.0g
Casamino acids .....	7.5g
Trisodium citrate .....	3.0g
KCl .....	2.0g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	36.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.36mg

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Haloarcula californiae*, *Haloarcula hispanica*, *Haloarcula japonica*, *Haloarcula marismortui*, *Haloarcula sinaiensis*, *Haloarcula vallismortis*, *Halobacterium cutirubrum*, *Halobacterium distributum*, *Halobacterium lacusprofundi*, *Halobacterium saccharovororum*, *Haloferax gibbonsii*, *Haloferax mediterranei*, *Halobacterium salinarum*, *Halobacterium simoncinii*, *Halobacterium* species, *Halobacterium trapanicum*, *Halobacterium volcanii*, *Halococcus morrhuae*, *Halococcus saccharolyticus*, *Halococcus* species, *Halococcus turkmenicus*, *Haloferax denitrificans*, *Halomonas elongata*, and *Salinicoccus roseus*.



**PB90-1 Medium  
(DSMZ Medium 298g)**

**Composition per liter:**

NaCl	1.0g
KCl	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.4g
NH <sub>4</sub> Cl	0.25g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	1.0mg
NaHCO <sub>3</sub> solution	10.0mL
Butanediol solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Vitamin solution	10.0mL
Seven vitamin solution	10.0mL
Glucose solution	10.0mL
Trace elements solution SL-10	1.0mL

pH 7.2 ± 0.2 at 25°C

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub>	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Butanediol Solution:****Composition per 10.0mL:**

2,3 butanediol	0.9g
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**Preparation of Butanediol Solution:** Add butanediol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg

p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Seven Vitamin Solution:****Composition per liter:**

Pyridoxine hydrochloride	300.0mg
Thiamine-HCl·2H <sub>2</sub> O	200.0mg
Nicotinic acid	200.0mg
Vitamin B <sub>12</sub>	100.0mg
Calcium pantothenate	100.0mg
p-Aminobenzoic acid	80.0mg
D-(+)-Biotin	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Glucose Solution:****Composition per 10.0mL:**

Glucose	0.7g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, butanediol solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, vitamin solution, seven vitamin solution, glucose solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 939.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL butanediol solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL 10.0mL vitamin solution, 10.0mL seven vitamin solution, 10.0mL glucose solution, trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% N<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Opitutus terrae*.

***Pectobacterium carotovorum* Medium  
(LMG Medium 172)**

**Composition per liter:**

Glucose	5.0g
Peptone	5.0g
Yeast extract	3.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pectobacterium carotovorum* subsp. *Odoriferum*.

***Pelobacter acetylenicus* Medium**

**Composition per liter:**

NaCl	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.0g

KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
NaHCO <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Acetoin solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### NaHCO<sub>3</sub> Solution:

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Acetoin Solution:

**Composition** per 10.0mL:

Acetoin.....	1.0g
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**Preparation of Acetoin Solution:** Add acetoin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and acetoin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile acetoin solution or, using a syringe, inject the appropriate amount of sterile NaHCO<sub>3</sub> solution, sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and sterile acetoin solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Pelobacter acetylenicus*.

#### *Pelobacter acidigallici* Medium

**Composition** per 1001.0mL:

NaCl.....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	3.0g
KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
NaHCO <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Gallic acid solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### NaHCO<sub>3</sub> Solution:

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Gallic Acid Solution:

**Composition** per 10.0mL:

Gallic acid.....	1.0g
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**Preparation of Gallic Acid Solution:** Add gallic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, gallic acid solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL of sterile gallic acid solution, and 1.0mL of sterile trace elements solution SL-10 or, using a syringe, inject the appropriate amount of sterile NaHCO<sub>3</sub> solution, sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, sterile gallic acid solution, and sterile trace elements solution SL-10 into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Pelobacter acidigallici*.

#### *Pelobacter carbinolicus* Medium

**Composition** per 1001.0mL:

NaCl .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
2,3-Butanediol solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### NaHCO<sub>3</sub> Solution:

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### 2,3-Butanediol Solution:

**Composition** per 10.0mL:

2,3-Butanediol .....	0.68g
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**Preparation of 2,3-Butanediol Solution:** Add 2,3-butanediol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, 2,3-butanediol solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL of sterile 2,3-butanediol solution, and 1.0mL of sterile trace elements solution SL-10 or, using a syringe, inject the appropriate amount of sterile

NaHCO<sub>3</sub> solution, sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, sterile 2,3-butanediol solution, and sterile trace elements solution SL-10 into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Pelobacter carbinolicus*.

#### *Pelobacter* Medium

**Composition** per 1025.0mL:

KHCO <sub>3</sub> .....	4.5g
NH <sub>4</sub> Cl .....	1.0g
NaCl .....	0.6g
Trypticase .....	0.5g
Yeast extract .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.08g
Resazurin .....	1.0mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
Sodium gallate solution .....	10.0mL
L-Cysteine·HCl·H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
pH 7.3 ± 0.2 at 25°C	

#### Trace Elements Solution:

**Composition** per liter:

Nitrilotriacetic acid .....	12.8g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.35g
NaCl .....	1.0g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.10g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.10g
ZnCl <sub>2</sub> .....	0.10g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.026g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.025g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### Vitamin Solution:

**Composition** per liter:

Biotin .....	2.0mg
Folic acid .....	2.0mg
Pyridoxine·HCl .....	10.0mg
Thiamine·HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Vitamin B <sub>12</sub> .....	0.1mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Sodium Gallate Solution:

**Composition** per 10.0mL:

Gallic acid .....	1.88g
NaOH (1M solution) .....	variable

**Preparation of Sodium Gallate Solution:** Add gallic acid to distilled/deionized water and bring volume to 8.0mL. Mix thoroughly. Add sufficient NaOH solution to

bring pH to 7.3. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### L-Cysteine-HCl-H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl-H<sub>2</sub>O ..... 0.3g

**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, sodium gallate solution, and vitamin solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL of sterile sodium gallate solution, and 10.0mL of sterile vitamin solution or, using a syringe, inject the appropriate amount of sterile NaHCO<sub>3</sub> solution, sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, sterile sodium gallate solution, and sterile vitamin solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Pelobacter acidigallici*.

#### *Pelobacter* Medium with Gallic Acid

**Composition** per liter:

Solution A ..... 950.0mL

Solution B ..... 25.0mL

Solution C ..... 25.0mL

pH 7.2 ± 0.2 at 25°C

#### Solution A:

**Composition** per liter:

NaCl ..... 20.0g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 3.65g

NaHCO<sub>3</sub> ..... 2.5g

KCl ..... 0.5g

NH<sub>4</sub>Cl ..... 0.25g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g

Resazurin ..... 0.5mg

Modified Wolfe's mineral solution ..... 10.0mL

Wolfe's vitamin solution ..... 10.0mL

**Preparation of Solution A:** Prepare and dispense solution under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub>, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Anaerobically distribute 9.5mL volumes into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C.

#### Modified Wolfe's Mineral Solution:

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g

Nitrilotriacetic acid ..... 1.5g

NaCl ..... 1.0g

MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.5g

CaCl<sub>2</sub> ..... 0.1g

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

AlK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.01g

CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g

H<sub>3</sub>BO<sub>3</sub> ..... 0.01g

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

Na<sub>2</sub>SeO<sub>3</sub> ..... 0.01g

NaWO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.01g

#### Preparation of Modified Wolfe's Mineral Solution:

Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

#### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl ..... 10.0mg

*p*-Aminobenzoic acid ..... 5.0mg

Lipoic acid ..... 5.0mg

Nicotinic acid ..... 5.0mg

Riboflavin ..... 5.0mg

Thiamine-HCl ..... 5.0mg

Calcium DL-pantothenate ..... 5.0mg

Biotin ..... 2.0mg

Folic acid ..... 2.0mg

Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution B:

**Composition** per 25.0mL:

Gallic acid ..... 0.85g

**Preparation of Solution B:** Prepare solution B immediately prior to use. Add gallic acid to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Rapidly adjust pH to 6.5. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### Solution C:

**Composition** per 25.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution C:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically add 0.25mL of sterile solution B and 0.25mL of sterile solution C to each tube containing 9.5mL of sterile solution A. Mix thoroughly. Adjust pH to 7.2.

**Use:** For the cultivation of *Pelobacter acidigallici* and *Pelobacter massiliensis*.

#### *Pelobacter propionicus* Medium (DSMZ Medium 298)

**Composition** per liter:

NaCl ..... 1.0g

KCl ..... 0.5g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.4g

NH<sub>4</sub>Cl ..... 0.25g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g

Resazurin ..... 1.0mg

NaHCO<sub>3</sub> solution ..... 10.0mL

Butanediol solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Butanediol Solution:****Composition per 10.0mL:**

2,3 butanediol.....	0.9g
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**Preparation of Butanediol Solution:** Add butanediol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, butanediol solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 969.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL butanediol solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% N<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Pelobacter propionicus*.

***Pelobacter venetianus* Marine Medium  
(DSMZ Medium 296)**

**Composition per liter:**

NaCl.....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	3.0g

KCl.....	0.5g
NH <sub>4</sub> Cl.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	1.0mg
NaHCO <sub>3</sub> solution.....	10.0mL
Polyethylene glycol solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL
pH 7.2 ± 0.2 at 25°C	

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Polyethylene Glycol Solution:****Composition per 10.0mL:**

Polyethylene glycol (molecular weight 106 - 20000).....	1.0g
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**Preparation of Polyethylene Glycol Solution:** Add polyethylene glycol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, polyethylene glycol solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 969.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL polyethylene glycol solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80% N<sub>2</sub> + 20% CO<sub>2</sub> to 1 bar overpressure.

**Use:** For the cultivation of *Pelobacter venetianus*.

***Pelotomaculum* Medium  
(DSMZ Medium 960)**

**Composition per liter:**

NaHCO <sub>3</sub> .....	2.5g
NH <sub>4</sub> Cl.....	0.54g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14g
Yeast extract.....	0.1g
Resazurin.....	0.5mg
Na-pyruvate solution.....	20.0mL
Cysteine solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Vitamin solution.....	5.0mL
Trace elements solution.....	1.0mL
Selenite-tungstate solution.....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Na-Pyruvate Solution:****Composition per 20mL:**

Na-pyruvate.....	2.2g
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**Preparation of Na-Pyruvate Solution:** Add Na-pyruvate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl.....	10.0mg
Thiamine·HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitritotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Selenite-Tungstate Solution****Composition per liter:**

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except Na-pyruvate solution, cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Equilibrate with this gas mixture to reach pH 7.0. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium 20.0mL sterile Na-pyruvate solution, 10.0mL sterile cysteine solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Pelotomaculum thermopropionicum*.

**Pentachlorophenol Medium**

**Composition per liter:**

NH <sub>4</sub> NO <sub>3</sub> .....	2.5g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Fe(SO <sub>4</sub> ) <sub>3</sub> ·5H <sub>2</sub> O.....	0.01g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.005g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.0mg
Pentachlorophenol.....	1.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	0.5mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.1mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	0.1mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Flavobacterium* species.

**Pentachlorophenol Medium**

**Composition per 1007.0mL:**

Sodium glutamate.....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.65g
NaNO <sub>3</sub> .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.19g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Pentachlorophenol solution.....	5.0mL
FeSO <sub>4</sub> solution.....	2.0mL

pH 7.3 ± 0.1 at 25°C

**Pentachlorophenol Solution:****Composition** per 100.0mL:

Pentachlorophenol .....	1.0g
NaOH (0.5 <i>N</i> solution) .....	100.0mL

**Preparation of Pentachlorophenol Solution:** Add pentachlorophenol to 100.0mL of NaOH solution. Mix thoroughly. Filter sterilize.

**FeSO<sub>4</sub> Solution:****Composition** per 100.0mL:

FeSO <sub>4</sub> solution .....	2.5g
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**Preparation of FeSO<sub>4</sub> Solution:** Add FeSO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except pentachlorophenol solution and FeSO<sub>4</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3–7.4. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 2.0mL of sterile FeSO<sub>4</sub> solution. Mix thoroughly. Aseptically distribute into sterile flasks. Inoculate flasks and place on a shaker at 200 rpm at 25°–30°C. Monitor growth with a spectrophotometer at 560 nm. When absorbance at 560 nm (*A*<sub>560</sub>) is 0.5, add 5.0mL of sterile pentachlorophenol solution per liter of medium.

**Use:** For the cultivation of *Pseudomonas mendocina*.

**Peptone Succinate Agar****Composition** per liter:

Agar .....	15.0g
Peptone.....	5.0g
Succinic acid .....	1.68g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Aquaspirillum bengal*, *Aquaspirillum dispar*, and *Spirillum volutans*.

**Peptone Succinate Agar****Composition** per liter:

Peptone.....	5.0g
Succinic acid .....	1.68g
Agar .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> SO <sub>4</sub> .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Aquaspirillum serpens*.

**Peptone Succinate Agar in Seawater****Composition** per liter:

Peptone.....	5.0g
Succinic acid .....	1.68g
Agar .....	1.5g

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	2.0mg
Seawater.....	1.0L

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Oceanospirillum maris*.

**Peptone Succinate Salts Broth  
(PSS Broth)****Composition** per 100.0mL:

Peptone.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
Succinic acid .....	0.1g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.2mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.2mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8 with KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Spirillum* species.

**Peptone Succinate Salts Medium  
(PSS Medium)****Composition** per liter:

Peptone.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Succinic acid .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	2.0mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add solid components to synthetic seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8 with 2*N* KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Aquaspirillum anulus*.

**Peptone Succinate Salts in Seawater****Composition** per liter:

Peptone.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Succinic acid .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	2.0mg
Synthetic seawater.....	1.0L

pH 6.8 ± 0.2 at 25°C

**Synthetic Seawater:****Composition** per liter:

NaCl .....	27.5g
MgCl <sub>2</sub> .....	5.0g
MgSO <sub>4</sub> .....	2.0g
KCl .....	1.0g
CaCl <sub>2</sub> .....	0.5g
FeSO <sub>4</sub> .....	1.0μg

**Preparation of Synthetic Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to synthetic seawater and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8 with 2N KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Oceanospirillum maris*.

### Peptone Sucrose Broth

**Composition** per liter:

Sucrose.....	20.0g
Peptone.....	10.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Xanthomonas campestris*.

### Peptone Yeast Extract Agar (ATCC Medium 526)

**Composition** per liter:

Agar .....	15.0g
Peptone.....	10.0g
Yeast extract.....	3.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bdellovibrio bacteriovorus* and *Bdellovibrio stolpii*.

### Peptone Yeast Extract Medium (ATCC Medium 1366)

**Composition** per liter:

Peptone.....	10.0g
NaCl .....	5.0g
Yeast extract.....	5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Xenorhabdus nematophilus*.

### Peptone Yeast Extract Medium (PY Medium) (ATCC Medium 1524)

**Composition** per 950.0mL:

Yeast extract.....	10.0g
Peptone.....	5.0g
Pancreatic digest of casein.....	5.0g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.5g
Salt solution .....	40.0mL
Hemin solution.....	10.0mL
Resazurin (0.025% solution).....	4.0mL
Vitamin K <sub>1</sub> solution .....	0.2mL

pH 7.0 ± 0.2 at 25°C

### Salt Solution:

**Composition** per liter:

NaHCO <sub>3</sub> .....	10.0g
NaCl .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
CaCl <sub>2</sub> , anhydrous.....	0.2g
MgSO <sub>4</sub> .....	0.2g

**Preparation of Salt Solution:** Add CaCl<sub>2</sub> and MgSO<sub>4</sub> to 300.0mL of distilled/deionized water. Mix thoroughly until dissolved. Bring volume to 800.0mL with distilled/deionized water. Add remaining components while stirring. Bring volume to 1.0L. Mix thoroughly. Store at 4°C.

### Hemin Solution:

**Composition** per 100.0mL:

Hemin .....	0.05g
NaOH (1N solution).....	1.0mL

**Preparation of Hemin Solution:** Add hemin to NaOH solution and bring volume to 100.0mL with distilled/deionized water. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

### Vitamin K<sub>1</sub> Solution:

**Composition** per 30.0mL:

Vitamin K <sub>1</sub> .....	0.15g
Ethanol (95% solution).....	30.0mL

**Preparation of Vitamin K<sub>1</sub> Solution:** Add Vitamin K<sub>1</sub> to ethanol. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Vitamin K<sub>1</sub> solution, hemin solution, and L-cysteine·HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 939.8mL. Gently heat and bring to boiling under 80% N<sub>2</sub> + 10% H<sub>2</sub> + 10% CO<sub>2</sub>. Continue boiling until resazurin turns colorless, indicating reduction. Cool to 45°–50°C. Add Vitamin K<sub>1</sub> solution, hemin solution, and L-cysteine·HCl·H<sub>2</sub>O. Adjust pH to 7.0. Distribute into tubes under 80% N<sub>2</sub> + 10% H<sub>2</sub> + 10% CO<sub>2</sub>. Cap with rubber stoppers. Place tubes in a press. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation and maintenance of *Megasphaera cerevisiae* and *Clostridium* species.

### Peptone Yeast Medium with Magnesium Sulfate (DSMZ Medium 790)

**Composition** per liter:

Peptone .....	10.0g
Yeast extract, dehydrated.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Aquaspirillum psychrophilum* and *Aquaspirillum peregrinum* subsp. *integrum*.

### Peptone Yeast Medium with MgSO<sub>4</sub>

**Composition** per liter:

Peptone .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
Yeast extract .....	1.0g

pH 7.0 ± 0.2 at 25°C



**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Aquaspirillum itersonii*, *Aquaspirillum peregrinum*, and *Aquaspirillum psychrophilum*.

### Peptonized Milk Agar (PMA Medium)

**Composition** per liter:

Agar .....	15.0g
Milk, peptonized .....	1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of freshwater *Myxobacterium* species.

### Petrotoxa Medium (ATCC 1881)

**Composition** per liter:

NaCl .....	20.0g
Sodium PIPES (piperazine- <i>N,N'</i> -bis[2-ethanesulfonic acid]) buffer .....	5.24g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	2.0g
Resazurin .....	1.0g
Soluble starch .....	1.0g
L-Cysteine-HCl-H <sub>2</sub> O .....	0.5g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except L-cysteine-HCl-H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.4. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Add L-cysteine-HCl-H<sub>2</sub>O. Mix thoroughly. Anaerobically distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Petrotoxa miotherma*.

### Pfennig's Medium I, Modified for Marine Purple Sulfur Bacteria (DSMZ Medium 28)

**Composition** per 5.0L:

Solution A .....	4.0L
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	20.0mL
Solution C .....	5.0mL
Solution D .....	5.0mL
pH 7.3 at 25°C	

**Solution A:**

**Composition** per 4.0L:

NaCl .....	100.0g
MgSO <sub>4</sub> .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
KCl .....	1.7g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g

**Preparation of Solution A:** Add components to 4.0L distilled water. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C in 5-liter special bottle or flask with 4

openings at the top, together with a teflon-coated magnetic bar. In this 5-liter bottle, 2 openings for tubes are in the central, silicon rubber stopper—a short, gas-inlet tube with a sterile cotton filter; and an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps—one of these openings is for the addition of sterile solutions, and the other serves as a gas outlet. After autoclaving, cool solution A to room temperature under a N<sub>2</sub> atmosphere with a positive pressure of 0.05–0.1 atm (a manometer for low pressure will be required). Saturate the cold medium with CO<sub>2</sub> by magnetic stirring for 30 min under a CO<sub>2</sub> atmosphere of 0.05–0.1 atm.

**Solution B:**

Distilled water .....	860.0mL
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**Preparation of Solution B:** Autoclave distilled water for 15 min at 15 psi pressure–121°C in a cotton-stoppered Erlenmeyer flask. Cool to room temperature under an atmosphere of N<sub>2</sub> in an anaerobic jar.

**Solution C:**

**Composition** per 100.0mL:

Vitamin B <sub>12</sub> .....	2.0mg
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**Preparation of Solution C:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

**Solution D:**

**Composition** per liter:

Disodium ethylenediamine-tetraacetate (Disodium EDTA) .....	3.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
ZnCl <sub>2</sub> .....	42.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	18.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

**Solution F:**

**Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Neutralized Sulfide Solution:**

**Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.5g
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**Preparation of Neutralized Sulfide Solution:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum, and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.3 with sterile 2M  $\text{H}_2\text{SO}_4$ . Do not open the bottle to add  $\text{H}_2\text{SO}_4$ ; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add solutions B, C, D, E, and F to solution A through one of the screw-cap openings against a stream of either  $\text{N}_2$  gas or better, a mixture of 95%  $\text{N}_2$  and 5%  $\text{CO}_2$  while the medium is magnetically stirred. Adjust the pH of the medium with sterile HCl or  $\text{Na}_2\text{CO}_3$  solution (2 M solutions) to pH 7.3. Distribute the medium aseptically through the medium outlet tube into sterile, 100mL bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05 - 0.1 atm) of the  $\text{N}_2/\text{CO}_2$  gas mixture. Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-capped bottles can be stored for several weeks or months in the dark. During the first 24 hr, the iron of the medium precipitates in the form of black flocs. No other sediment should arise in the otherwise clear medium. Incubate in the light using a tungsten lamp. Feed periodically with neutralized solution of sodium sulfide to replenish sulfide with other supplement solutions.

**Use:** For the cultivation of marine purple sulfur bacteria.

### Pfennig's Medium I, Modified for Purple Sulfur Bacteria (DSMZ Medium 28)

#### Composition per 5.0L:

Solution A .....	4.0L
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	20.0mL
Solution C .....	5.0mL
Solution D .....	5.0mL

pH 7.3 at 25°C

#### Solution A:

##### Composition per 4.0L:

$\text{MgSO}_4$ .....	2.5g
$\text{KH}_2\text{PO}_4$ .....	1.7g
$\text{NH}_4\text{Cl}$ .....	1.7g
KCl .....	1.7g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	1.25g

**Preparation of Solution A:** Add components to 4.0L distilled water. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C in 5-liter special bottle or flask with 4 openings at the top, together with a teflon-coated magnetic bar. In this 5-liter bottle, 2 openings for tubes are in the central, silicon rubber stopper—a short, gas-inlet tube with a sterile cotton filter; and an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps—one of these openings is for the addition of sterile solutions, and the other serves as a gas outlet. After autoclaving, cool solution A to room temperature under a  $\text{N}_2$  atmosphere with a positive pressure of 0.05–0.1 atm (a manometer for low pressure will be required). Saturate the cold medium with  $\text{CO}_2$  by

magnetic stirring for 30 min under a  $\text{CO}_2$  atmosphere of 0.05–0.1 atm.

#### Solution B:

Distilled water ..... 860.0mL

**Preparation of Solution B:** Autoclave distilled water for 15 min at 15 psi pressure–121°C in a cotton-stoppered Erlenmeyer flask. Cool to room temperature under an atmosphere of  $\text{N}_2$  in an anaerobic jar.

#### Solution C:

##### Composition per 100.0mL:

Vitamin  $\text{B}_{12}$  ..... 2.0mg

**Preparation of Solution C:** Add Vitamin  $\text{B}_{12}$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Filter sterilize. Store under  $\text{N}_2$  gas.

#### Solution D:

##### Composition per liter:

Disodium ethylenediamine-tetraacetate (Disodium EDTA) .....	3.0g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	1.1g
$\text{H}_3\text{BO}_3$ .....	0.3g
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.19g
$\text{MnCl}_2\cdot 2\text{H}_2\text{O}$ .....	50.0mg
$\text{ZnCl}_2$ .....	42.0mg
$\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ .....	24.0mg
$\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ .....	18.0mg
$\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ .....	2.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

##### Composition per 100.0mL:

$\text{NaHCO}_3$  ..... 7.5g

**Preparation of Solution E:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100%  $\text{CO}_2$  until saturated. Filter sterilize under 100%  $\text{CO}_2$  into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution F:

##### Composition per 100.0mL:

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  ..... 10.0g

**Preparation of Solution F:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Neutralized Sulfide Solution:

##### Composition per 100.0mL:

$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  ..... 1.5g

**Preparation of Neutralized Sulfide Solution:** Add  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.3 with sterile 2M  $\text{H}_2\text{SO}_4$ . Do not open the bottle to add  $\text{H}_2\text{SO}_4$ ; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add solutions B, C, D, E, and F to solution A through one of the screw-cap openings against a stream of either N<sub>2</sub> gas or better, a mixture of 95% N<sub>2</sub> and 5% CO<sub>2</sub> while the medium is magnetically stirred. Adjust the pH of the medium with sterile HCl or Na<sub>2</sub>CO<sub>3</sub> solution (2 M solutions) to pH 7.3. Distribute the medium aseptically through the medium outlet tube into sterile, 100mL bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05–0.1 atm) of the N<sub>2</sub>/CO<sub>2</sub> gas mixture. Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-capped bottles can be stored for several weeks or months in the dark. During the first 24 hr, the iron of the medium precipitates in the form of black flocs. No other sediment should arise in the otherwise clear medium. Incubate in the light using a tungsten lamp. Feed periodically with neutralized solution of sodium sulfide to replenish sulfide with other supplement solutions.

**Use:** For the cultivation of purple sulfur bacteria.

#### **Pfennig's Medium 1 with 1% Salt**

##### **Composition per 4990.0mL:**

Solution A .....	4000.0mL
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	20.0mL
Solution C (Vitamin B <sub>12</sub> solution) .....	5.0mL
Solution D (Trace elements solution SL-12B) .....	5.0mL
pH 7.3 ± 0.2 at 25°C	

##### **Solution A:**

##### **Composition per 4000.0mL:**

NaCl .....	50.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
KCl .....	1.7g
MgSO <sub>4</sub> .....	2.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Adjust pH to 6.0. Dispense into a 5-liter flask with 4 openings at the top (two openings are in a central silicon rubber stopper and 2 openings are gas-tight screw caps). Add a teflon-coated magnetic stir bar to the flask. Autoclave for 45 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> at 0.05–0.1 atm pressure (use a manometer to measure low pressure).

##### **Solution B:**

##### **Composition per 860.0mL:**

Distilled/deionized water .....	860.0mL
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**Preparation of Solution B:** Add 860.0mL of distilled/deionized water to a cotton-stoppered flask. Autoclave for 20 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> in an anaerobic jar.

##### **Solution C (Vitamin B<sub>12</sub> Solution):**

##### **Composition per 5.0mL:**

Vitamin B <sub>12</sub> .....	1.0mg
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**Preparation of Solution C (Vitamin B<sub>12</sub> Solution):** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Filter sterilize.

##### **Solution D (Trace Elements Solution SL-12B):**

##### **Composition per liter:**

Disodium ethylenediamine-tetraacetate (Na <sub>2</sub> -EDTA) .....	3.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
ZnCl <sub>2</sub> .....	42.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	18.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg

**Preparation of Solution D (Trace Elements Solution SL-12B):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

##### **Solution E:**

##### **Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

##### **Solution F:**

##### **Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Dispense into a screw-capped bottle. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Saturate cooled solution A under 100% CO<sub>2</sub> at 0.05–0.1 atm pressure for 30 min with magnetic stirring. Add 860.0mL of solution B, 5.0mL of solution C, 5.0mL of solution D, 100.0mL of solution E, and 20.0mL of solution F through one of the screw-capped openings under 95% N<sub>2</sub> and 5% CO<sub>2</sub> with magnetic stirring. Adjust pH to 7.3 with sterile 2M HCl or sterile 2M Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically and anaerobically distribute the medium through the medium outlet tube into sterile 100.0mL bottles under 95% N<sub>2</sub> and 5% CO<sub>2</sub> at 0.05–0.1 atm pressure. Leave a small gas bubble in each bottle to accommodate pressure changes. After 24 hr, the iron in the medium will precipitate out of solution as black flocs.

**Use:** For the cultivation and maintenance of *Ectothiorhodospira mobilis*.

#### **Pfennig's Medium 1 with 3% Salt**

##### **Composition per 4990.0mL:**

Solution A .....	4000.0mL
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	20.0mL
Solution C (Vitamin B <sub>12</sub> solution) .....	5.0mL
Solution D (Trace elements solution SL-12B) .....	5.0mL
pH 7.3 ± 0.2 at 25°C	

##### **Solution A:**

##### **Composition per 4000.0mL:**

NaCl .....	150.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
KCl .....	1.7g
MgSO <sub>4</sub> .....	2.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Adjust pH to 6.0. Dispense into a 5-liter flask with 4 openings at the top (2 openings are in a central silicone

rubber stopper and 2 openings are gas-tight screw caps). Add a teflon-coated magnetic stir bar to the flask. Autoclave for 45 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> at 0.05–0.1 atm pressure (use a manometer to measure low pressure).

#### Solution B:

##### Composition per 860.0mL:

Distilled/deionized water ..... 860.0mL

**Preparation of Solution B:** Add 860.0mL of distilled/deionized water to a cotton-stoppered flask. Autoclave for 20 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> in an anaerobic jar.

#### Solution C (Vitamin B<sub>12</sub> Solution):

##### Composition per 5.0mL:

Vitamin B<sub>12</sub> ..... 1.0mg

**Preparation of Solution C (Vitamin B<sub>12</sub> Solution):** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Filter sterilize.

#### Solution D (Trace Elements Solution SL-12B):

##### Composition per liter:

Disodium ethylenediamine-tetraacetate  
(Na<sub>2</sub>-EDTA) ..... 3.0g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.1g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
MnCl<sub>2</sub>·2H<sub>2</sub>O ..... 50.0mg  
ZnCl<sub>2</sub> ..... 42.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 18.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg

**Preparation of Solution D (Trace Elements Solution SL-12B):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

##### Composition per 100.0mL:

NaHCO<sub>3</sub> ..... 7.5g

**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution F:

##### Composition per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 10.0g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Dispense into a screw-capped bottle. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Saturate cooled solution A under 100% CO<sub>2</sub> at 0.05–0.1 atm pressure for 30 min with magnetic stirring. Add 860.0mL of solution B, 5.0mL of solution C, 5.0mL of solution D, 100.0mL of solution E, and 20.0mL of solution F through one of the screw-capped openings under 95% N<sub>2</sub> and 5% CO<sub>2</sub> with magnetic stirring. Adjust pH to 7.3 with sterile 2M HCl or sterile 2M Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically and anaerobically distribute the medium through the medium outlet tube into sterile 100.0mL bottles under 95% N<sub>2</sub> and 5% CO<sub>2</sub> at 0.05–0.1 atm pressure. Leave a small gas bubble in each bottle to accommodate pressure changes. After 24 hr, the iron in the medium will precipitate out of solution as black flocs.

**Use:** For the cultivation and maintenance of *Chromatium gracile*, *Thiocystis gelatinosa*, and *Thiocystis violacea*.

#### Pfennig's Medium 1 with Yeast Extract

##### Composition per 4990.0mL:

Solution A ..... 4000.0mL  
Solution B ..... 860.0mL  
Solution E ..... 100.0mL  
Solution F ..... 20.0mL  
Solution C (Vitamin B<sub>12</sub> solution) ..... 5.0mL  
Solution D (Trace elements solution SL-12B) ..... 5.0mL  
pH 7.3 ± 0.2 at 25°C

#### Solution A:

##### Composition per 4000.0mL:

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.25g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.7g  
NH<sub>4</sub>Cl ..... 1.7g  
KCl ..... 1.7g  
MgSO<sub>4</sub> ..... 2.5g  
Yeast extract ..... 2.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Adjust pH to 6.0. Dispense into a 5-liter flask with 4 openings at the top (2 openings are in a central silicon rubber stopper and 2 openings are gas-tight screw caps). Add a teflon-coated magnetic stir bar to the flask. Autoclave for 45 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> at 0.05–0.1 atm pressure (use a manometer to measure low pressure).

#### Solution B:

##### Composition per 860.0mL:

Distilled/deionized water ..... 860.0mL

**Preparation of Solution B:** Add 860.0mL of distilled/deionized water to a cotton-stoppered flask. Autoclave for 20 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> in an anaerobic jar.

#### Solution C (Vitamin B<sub>12</sub> Solution):

##### Composition per 5.0mL:

Vitamin B<sub>12</sub> ..... 1.0mg

#### Preparation of Solution C (Vitamin B<sub>12</sub> Solution):

Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Filter sterilize.

#### Solution D (Trace Elements Solution SL-12B):

##### Composition per liter:

Disodium ethylenediamine-tetraacetate  
(Na<sub>2</sub>-EDTA) ..... 3.0g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.1g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
MnCl<sub>2</sub>·2H<sub>2</sub>O ..... 50.0mg  
ZnCl<sub>2</sub> ..... 42.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 18.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg

#### Preparation of Solution D (Trace Elements Solution SL-12B):

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

##### Composition per 100.0mL:

NaHCO<sub>3</sub> ..... 7.5g

**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thor-

oughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution F:

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 10.0g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Dispense into a screw-capped bottle. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Saturate cooled solution A under 100% CO<sub>2</sub> at 0.05–0.1 atm pressure for 30 min with magnetic stirring. Add 860.0mL of solution B, 5.0mL of solution C, 5.0mL of solution D, 100.0mL of solution E, and 20.0mL of solution F through one of the screw-capped openings under 95% N<sub>2</sub> and 5% CO<sub>2</sub> with magnetic stirring. Adjust pH to 7.3 with sterile 2M HCl or sterile 2M Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically and anaerobically distribute the medium through the medium outlet tube into sterile 100.0mL bottles under 95% N<sub>2</sub> and 5% CO<sub>2</sub> at 0.05–0.1 atm pressure. Leave a small gas bubble in each bottle to accommodate pressure changes. After 24 hr, the iron in the medium will precipitate out of solution as black flocs.

**Use:** For the cultivation and maintenance of *Amoebobacter pendens*.

### Pfennig's Medium II Modified for Green Sulfur Bacteria (DSMZ Medium 29)

**Composition** per 5.0L:

Solution A ..... 4.0L  
Solution B ..... 860.0mL  
Solution E ..... 100.0mL  
Solution F ..... 30.0mL  
Solution C ..... 5.0mL  
Solution D ..... 5.0mL

pH 6.8 at 25°C

#### Solution A:

**Composition** per 4.0L:

MgSO<sub>4</sub> ..... 2.5g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.7g  
NH<sub>4</sub>Cl ..... 1.7g  
KCl ..... 1.7g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.25g

**Preparation of Solution A:** Add components to 4.0L distilled water. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C in 5-liter special bottle or flask with 4 openings at the top, together with a teflon-coated magnetic bar. In this 5-liter bottle, 2 openings for tubes are in the central, silicone rubber stopper, a short, gas-inlet tube with a sterile cotton filter—and an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps—one of these openings is for the addition of sterile solutions and the other serves as a gas outlet. After autoclaving, cool solution A to room temperature under a N<sub>2</sub> atmosphere with a positive pressure of 0.05–0.1 atm (a manometer for low pressure will be required). Saturate the cold medium with CO<sub>2</sub> by magnetic stirring for 30 min under a CO<sub>2</sub> atmosphere of 0.05–0.1 atm.

#### Solution B:

Distilled water ..... 860.0mL

**Preparation of Solution B:** Autoclave distilled water for 15 min at 15 psi pressure–121°C in a cotton-stoppered Erlenmeyer flask. Cool to room temperature under an atmosphere of N<sub>2</sub> in an anaerobic jar.

#### Solution C:

**Composition** per 100.0mL:

Vitamin B<sub>12</sub> ..... 2.0mg

**Preparation of Solution C:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

#### Solution D:

**Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 300.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 7.7mL

**Preparation of Solution D:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 7.5g

**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution F:

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 10.0g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Neutralized Sulfide Solution:

**Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 1.5g

**Preparation of Neutralized Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 6.8 with sterile 2M H<sub>2</sub>SO<sub>4</sub>. Do not open the bottle to add H<sub>2</sub>SO<sub>4</sub>; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add solutions B, C, D, E, and F to solution A through one of the screw-cap openings against a stream of either N<sub>2</sub> gas or better, a mixture of 95% N<sub>2</sub> and 5% CO<sub>2</sub> while the medium is magnetically stirred. Adjust the pH of the medium with sterile HCl or Na<sub>2</sub>CO<sub>3</sub>.

solution (2 M solutions) to pH 6.8. Distribute the medium aseptically through the medium outlet tube into sterile, 100mL bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05–0.1 atm) of the N<sub>2</sub>/CO<sub>2</sub> gas mixture. Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-capped bottles can be stored for several weeks or months in the dark. During the first 24 hr, the iron of the medium precipitates in the form of black flocs. No other sediment should arise in the otherwise clear medium. Incubate in the light using a tungsten lamp. Feed periodically with neutralized solution of sodium sulfideto replenish sulfide with other supplement solutions.

**Use:** For the cultivation of green sulfur bacteria.

### Pfennig's Medium II with Salt

**Composition per 5000.0mL:**

Solution A .....	4000.0mL
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	30.0mL
Solution C (Vitamin B <sub>12</sub> solution) .....	5.0mL
Solution D (Trace elements solution SL-10B).....	5.0mL
pH 6.8 ± 0.2 at 25°C	

### Solution A:

**Composition per 4000.0mL:**

NaCl .....	50.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.25g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
KCl .....	1.7g
MgSO <sub>4</sub> .....	2.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 4.0L. Mix thoroughly. Adjust pH to 6.0. Dispense into a 5-liter flask with 4 openings at the top (2 openings are in a central silicon rubber stopper and 2 openings are gas-tight screw caps). Add a teflon-coated magnetic stir bar to the flask. Autoclave for 45 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> at 0.05–0.1 atm pressure (use a manometer to measure low pressure).

### Solution B:

**Composition per 860.0mL:**

Distilled/deionized water .....	860.0mL
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**Preparation of Solution B:** Add 860.0mL of distilled/deionized water to a cotton-stoppered flask. Autoclave for 20 min at 15 psi pressure–121°C. Cool to room temperature under 100% N<sub>2</sub> in an anaerobic jar.

### Solution C (Vitamin B<sub>12</sub> Solution):

**Composition per 5.0mL:**

Vitamin B <sub>12</sub> .....	1.0mg
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### Preparation of Solution C (Vitamin B<sub>12</sub> Solution):

Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 5.0mL. Mix thoroughly. Filter sterilize.

### Solution D (Trace Elements Solution SL-10B):

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution D (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

### Solution E:

**Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> until saturated. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

### Solution F:

**Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	10.0g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Dispense into a screw-capped bottle. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Saturate cooled solution A under 100% CO<sub>2</sub> at 0.05–0.1 atm pressure for 30 min with magnetic stirring. Add 860.0mL of solution B, 5.0mL of solution C, 5.0mL of solution D, 100.0mL of solution E, and 20.0mL of solution F through one of the screw-capped openings under 95% N<sub>2</sub> and 5% CO<sub>2</sub> with magnetic stirring. Adjust pH to 6.8 with sterile 2M HCl or sterile 2M Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically and anaerobically distribute the medium through the medium outlet tube into sterile 100.0mL bottles under 95% N<sub>2</sub> and 5% CO<sub>2</sub> at 0.05–0.1 atm pressure. Leave a small gas bubble in each bottle to accommodate pressure changes. After 24 hr, the iron in the medium will precipitate out of solution as black flocs.

**Use:** For the cultivation and maintenance of *Chlorobium phaeobacteroides*, *Chlorobium vibrioforme*, *Pelodictyon luteolum*, *Pelodictyon phaeum*, and *Prosthecochloris aestuarii*.

### PFS Medium

(Peptone Fumarate Sulfate Medium)

**Composition per liter:**

Peptone .....	10.0g
Fumaric acid .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.2mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.2mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with KOH. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Aquaspirillum fasciculans*.

### Phenol Red Agar

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	10.0g
NaCl .....	5.0g

Phenol Red .....	0.018g
Carbohydrate solution .....	20.0mL
pH $7.4 \pm 0.2$ at $25^{\circ}\text{C}$	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

#### **Carbohydrate Solution:**

<b>Composition</b> per 20.0mL:	
Carbohydrate .....	5.0–10.0g

**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 7.4 if necessary. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ . Cool to  $45^{\circ}\text{C}$ – $50^{\circ}\text{C}$ . Aseptically add 20.0mL of sterile carbohydrate solution. Pour into sterile Petri dishes or distribute into sterile tubes. Allow tubes to cool in a slanted position.

**Use:** For the determination of fermentation reactions. Bacteria that can ferment the added carbohydrate turn the medium yellow.

#### **Phenol Red Glucose Broth**

##### **Composition** per liter:

Pancreatic digest of casein .....	10.0g
Glucose .....	5.0g
NaCl .....	5.0g
Phenol Red .....	0.018g
pH $7.3 \pm 0.2$ at $25^{\circ}\text{C}$	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3 if necessary. Distribute into tubes containing an inverted Durham tube. Fill each tube with 10.0mL of medium. Autoclave for 15 min at 13 psi pressure– $118^{\circ}\text{C}$ .

**Use:** For determination of the ability of a microorganism to ferment glucose. Fermentation is determined by the production of acid (when broth turns yellow) and formation of gas (when bubble trapped in Durham tube).

#### **Phenol Red Lactose Agar**

##### **Composition** per liter:

Agar .....	15.0g
Lactose .....	10.0g
Proteose peptone No. 3 .....	10.0g
NaCl .....	5.0g
Beef extract .....	1.0g
Phenol Red .....	25.0mg
pH $7.4 \pm 0.2$ at $25^{\circ}\text{C}$	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 13 psi pressure– $118^{\circ}\text{C}$ . Pour into sterile Petri dishes or leave in tubes. Allow tubes to cool in a slanted position.

**Use:** For determination of the ability of a microorganism to ferment lactose. Fermentation is determined by the production of acid (when medium turns yellow).

#### **Phenol Red Mannitol Agar**

##### **Composition** per liter:

Agar .....	15.0g
Mannitol .....	10.0g
Proteose peptone No. 3 .....	10.0g
NaCl .....	5.0g
Beef extract .....	1.0g
Phenol Red .....	25.0mg
pH $7.4 \pm 0.2$ at $25^{\circ}\text{C}$	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 13 psi pressure– $118^{\circ}\text{C}$ . Pour into sterile Petri dishes or leave in tubes. Allow tubes to cool in a slanted position.

**Use:** For determination of the ability of a microorganism to ferment mannitol. Fermentation is determined by the production of acid (when medium turns yellow).

#### **Phenol Red Sucrose Broth**

##### **Composition** per liter:

Pancreatic digest of casein .....	10.0g
NaCl .....	5.0g
Sucrose .....	5.0g
Phenol Red .....	0.018g
pH $7.3 \pm 0.2$ at $25^{\circ}\text{C}$	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3 if necessary. Distribute into tubes containing an inverted Durham tube. Fill each tube with 10.0mL of medium. Autoclave for 15 min at 13 psi pressure– $118^{\circ}\text{C}$ .

**Use:** For determination of the ability of a microorganism to ferment sucrose. Fermentation is determined by the production of acid (when broth turns yellow) and formation of gas (when bubble trapped in Durham tube).

#### **Phosphate Mineral Salts Medium with Octane**

##### **Composition** per liter:

$(\text{NH}_4)_2\text{HPO}_4$ .....	10.0g
$\text{K}_2\text{HPO}_4$ .....	5.0g
$\text{Na}_2\text{SO}_4$ .....	0.5g
Octane .....	10.0mL

**Preparation of Medium:** Add components, except octane, to tap water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ . Prior to inoculation, filter sterilize octane. Aseptically add sterile octane to sterile medium. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudomonas oleovorans*.

#### **Photobacterium Agar (DSMZ Medium 32)**

##### **Composition** per liter:

Seawater aquarium salt .....	33.0g
Agar .....	20.0g
Tris .....	6.0g
Yeast extract .....	5.0g
Tryptone .....	5.0g

NH <sub>4</sub> Cl.....	5.0g
Glycerol .....	3.0g
CaCO <sub>3</sub> .....	1.0g
pH 7.2-7.5 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2 - 7.5. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Vibrio fischeri*.

#### Photobacterium Broth

**Composition** per liter:

NaCl .....	30.0g
Sodium glycerol phosphate.....	23.5g
Pancreatic digest of casein .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract.....	2.5g
CaCO <sub>3</sub> .....	1.0g
NH <sub>4</sub> Cl.....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
FeCl <sub>3</sub> .....	0.01g
pH 7.0 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks to form a shallow layer of medium. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation and demonstration of luminescence by photobacteria. For the cultivation and maintenance of *Alteromonas hanedai*, *Photobacterium phosphoreum*, *Shewanella hanedai*, *Vibrio fischeri*, *Vibrio harveyi*, and other *Vibrio* species.

#### Photobacterium MPY Medium

**Composition** per liter:

NaCl .....	28.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.9g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.5g
Peptone.....	5.0g
Yeast extract.....	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.5g
KCl.....	0.7g
pH 7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation and maintenance of *Photobacterium leiognathi*.

#### Phthalic Acid Medium

**Composition** per liter:

Solution 1 .....	400.0mL
Solution 2 .....	400.0mL
Potassium hydrogen phthalate solution .....	200.0mL
pH 6.8 ± 0.2 at 25°C	

**Solution 1:**

**Composition** per 400.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	9.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.2g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 6.8 with KOH. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 25°C.

**Solution 2:**

**Composition** per 400.0mL:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix thoroughly. Adjust pH to 6.8 with KOH. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 25°C.

**Potassium Hydrogen Phthalate Solution:**

**Composition** per 200.0mL:

Potassium hydrogen phthalate.....	1.0g
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**Preparation of Medium:** Add component to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Adjust pH to 6.8 with KOH. Autoclave for 15 min at 15 psi pressure-121°C. Cool to 25°C.

**Preparation of Medium:** Aseptically combine the three sterile solutions. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudomonas cepacia*.

#### Pilobolus Agar

**Composition** per liter:

Agar .....	15.0g
Sodium acetate .....	10.0g
Yeast extract .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.66g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Thiamine.....	10.0mg
Hemin.....	10.0mg
NaOH (0.1N solution).....	37.5mL
pH 8.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add hemin to NaOH solution and mix thoroughly to dissolve. Add remaining components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pilobolus* species.

#### Pilobolus Medium (DSMZ Medium 192)

**Composition** per liter:

Agar .....	15.0g
Na-acetate.....	10.0g
Yeast extract .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.66g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Thiamine-HCl·2H <sub>2</sub> O .....	10.0mg
Haemin solution .....	37.5 mL
pH 8.0 ± 0.2 at 25°C	

**Haemin Solution:**

**Composition** per 10.0mL:

Haemin .....	10.0mg
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**Preparation of Haemin Solution:** Add haemin to 37.5 mL 0.1N NaOH. Mix thoroughly.



**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pilobolus sphaerosporus*.

#### *Pirellula marina* Medium M14

**Composition per liter:**

Glucose .....	1.0g
Yeast extract .....	1.0g
Artificial seawater .....	680.0mL
Tris-HCl buffer, (0.1M solution, pH 7.5) .....	50.0mL
Hutner's basal salts .....	20.0mL
Vitamin solution .....	10.0mL
pH 7.5 ± 0.2 at 25°C	

#### Artificial Seawater:

**Composition per liter:**

NaCl .....	23.47g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.64g
Na <sub>2</sub> SO <sub>4</sub> .....	3.92g
CaCl <sub>2</sub> .....	1.1g
KCl .....	664.0mg
NaHCO <sub>3</sub> .....	192.0mg
KBr .....	96.0mg
H <sub>3</sub> BO <sub>3</sub> .....	26.0mg
SnCl <sub>2</sub> .....	24.0mg
NaF .....	3.0mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

**Composition per liter:**

Nicotinamide .....	9.0mg
Calcium DL-pantothenate .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Pirellula marina*.

#### Plaice Medium

**Composition per liter:**

Fresh plaice, minced .....	200.0g
NaCl .....	120.0g
Peptone .....	20.0g
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Soak plaice in 4.0L of water and allow to stand for 2 hr. Boil for 1 hr and filter. Add peptone and NaCl. Adjust pH. Boil for a few minutes and filter. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of bioluminescent bacteria.

#### *Planctomyces* Medium

**Composition per liter:**

Glucose .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.25g
Peptone .....	0.15g
Yeast extract .....	0.15g
Seawater, aged filtered .....	1.0L

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Planctomyces* species.

#### Plant *Mycoplasma* Agar

**Composition per 291.2mL:**

Schneider's <i>Drosophila</i> medium .....	160.0mL
Solution 1 .....	70.0mL
Fetal calf serum .....	50.0mL
Fresh yeast extract solution .....	10.0mL
Phenol Red (0.5% solution) .....	1.2mL
pH 7.4 ± 0.2 at 25°C	

#### Schneider's *Drosophila* Medium:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.7g
NaCl .....	2.1g
Yeast extract .....	2.0g
Trehalose .....	2.0g
D-Glucose .....	2.0g
L-Glutamine .....	1.8g
L-Lysine-HCl .....	1.7g
L-Proline .....	1.7g
KCl .....	1.6g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	1.3g
L-Glutamic acid .....	0.8g
L-Methionine .....	0.8g
CaCl <sub>2</sub> , anhydrous .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
β-Alanine .....	0.5g
L-Tyrosine .....	0.5g
L-Arginine .....	0.4g
L-Aspartic acid .....	0.4g
L-Histidine .....	0.4g
L-Threonine .....	0.4g
NaHCO <sub>3</sub> .....	0.4g
Glycine .....	0.3g
L-Serine .....	0.3g
L-Valine .....	0.3g
L-Isoleucine .....	0.2g
L-Leucine .....	0.2g
L-Phenylalanine .....	0.2g
α-Ketoglutaric acid .....	0.2g
Fumaric acid .....	0.1g
Malic acid .....	0.1g
Succinic acid .....	0.1g
L-Cystine .....	0.1g
L-Tryptophan .....	0.1g
L-Cysteine .....	0.06g

**Preparation of Schneider's *Drosophila* Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Solution 1:

**Composition per 70.0mL:**

Sorbitol .....	7.0g
Noble agar .....	5.0g

Beef heart, solids from infusion	5.0g
Peptone	1.8g
Pancreatic digest of casein	1.0g
Sucrose	1.0g
NaCl	0.5g
D-Fructose	0.1g
D-Glucose	0.1g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Adjust pH to 7.8 with 1N NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

#### Fresh Yeast Extract Solution:

**Composition** per 100.0mL:

Baker's yeast, live, pressed, starch-free	25.0g
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**Preparation of Fresh Yeast Extract Solution:** Add the live Baker's yeast to 100.0mL of distilled/deionized water. Autoclave for 90 min at 15 psi pressure–121°C. Allow to stand. Remove supernatant solution. Adjust pH to 6.6–6.8.

**Preparation of Medium:** Bring fetal calf serum and Phenol Red solution to 56°C. Rapidly bring Schneider's *Drosophila* medium to 37°C. Rapidly combine the components. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma floricola*, *Spiroplasma kunkelii*, *Spiroplasma melliferum*, and *Spiroplasma* species.

#### Plates with Fluoranthene (DSMZ Medium 462a)

**Composition** per 1052mL:

Agar	15.0g
Na <sub>2</sub> HPO <sub>4</sub>	2.44g
KH <sub>2</sub> PO <sub>4</sub>	1.52g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Fluoranthene solution	50.0mL
Trace elements solution SL-4	10.0mL
Vitamin solution	2.5mL

pH 7.1 ± 0.2 at 25°C

#### Trace Elements Solution SL-4:

**Composition** per liter:

EDTA	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Trace elements solution SL-6	100.0mL

#### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

**Composition** per liter:

Pyridoxamine	5.0mg
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Vitamin B <sub>12</sub>	2.0mg
Nicotinic acid	2.0mg
p-Aminobenzoate	1.0mg
Thiamine-HCl·2H <sub>2</sub> O	1.0mg
Ca-pantothenate	0.5mg
Biotin	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Fluoranthene Solution:

**Composition** per 100.0mL:

Fluoranthene	10.0g
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**Preparation of Fluoranthene Solution:** Add fluoranthene to 100.0mL acetone. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution and fluoranthene solution, to 1.0L distilled/deionized water. Adjust pH to 7.1. Gently heat and bring to boil. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes (20.0mL per Petri dish). Allow plates to dry for 48 hr. Aseptically spread 1.0mL of fluoranthene solution onto the surface of the dried plates. Allow the acetone to evaporate under sterile condition.

**Use:** For the cultivation of *Mycobacterium gilvum*, *Sphingomonas* sp. (*Sphingomonas paucimobilis*), unclassified bacterium (*Gordona* sp.), *Mycobacterium* sp., *Sphingomonas* sp., and other biphenyl utilizing bacteria.

#### PMY Medium

**Composition** per liter:

Agar	15.0g
Glucose	10.0g
NaCl	5.0g
Polypeptone	5.0g
Beef extract	2.0g
Yeast extract	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas campestris*.

#### Postgate's Medium

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.0g
CaSO <sub>4</sub>	1.0g
NH <sub>4</sub> Cl	1.0g
Yeast extract	1.0g
K <sub>2</sub> HPO <sub>4</sub>	0.5g
Sodium lactate (70% solution)	3.5mL

**Preparation of Medium:** Add components, except FeSO<sub>4</sub>·7H<sub>2</sub>O, ascorbic acid, and thioglycollic acid, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 10–15 min. Add FeSO<sub>4</sub>·7H<sub>2</sub>O, ascorbic acid, and thioglycollic acid. Mix thoroughly. Continue to sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> and adjust pH to 7.4. Anaerobically distribute into tubes or flasks. Autoclave for 10 min at 10 psi pressure–115°C.

**Use:** For the cultivation of *Desulfobulbus propionicus*, *Desulfotomaculum nigrificans*, *Desulfotomaculum orientis*,

*Desulfotomaculum ruminis*, *Desulfovibrio africanus*, *Desulfovibrio desulfuricans*, *Desulfovibrio gigas*, *Desulfovibrio multispirans*, *Desulfovibrio* species, and *Desulfovibrio vulgaris*.

#### Potato P-YE *Thermus* Medium

##### Composition per liter:

Agar .....	20.0g
Peptone.....	5.0g
Yeast extract.....	0.2g
Potatoes, infusion from.....	200.0mL
pH 7.8 ± 0.2 at 25°C	

##### Potatoes, Infusion From:

##### Composition per 500.0mL:

Potatoes.....	300.0g
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**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermus ruber*.

#### PP Medium

##### Composition per liter:

Proteose peptone.....	10.0g
Pancreatic digest of peptone.....	10.0g
Ribonucleic acid from <i>Torula</i> yeast.....	1.0g
Asolectin.....	0.2g
Artificial seawater.....	167.0mL
Vitamin solution.....	2.0mL

##### Artificial Seawater:

##### Composition per 167.0mL:

Aqua-Marin sea salts.....	6.95g
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**Source:** Aqua-Marin sea salts are available from Aquatrol, Inc., Anaheim, CA.

**Preparation of Artificial Seawater:** Add Aqua-Marin sea salts to distilled/deionized water and bring volume to 167.0mL. Mix thoroughly. Filter sterilize.

##### Vitamin Solution:

##### Composition per 100.0mL:

Thiamine-HCl.....	150.0mg
Calcium D-(+)-pantothenate.....	100.0mg
Folic acid.....	50.0mg
Nicotinamide.....	50.0mg
Pyridoxal-HCl.....	50.0mg
Riboflavin.....	50.0mg
DL-6 Thioctic acid.....	1.0mg
Biotin solution.....	10.0mL

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. For long-term storage, preserve under nitrogen at –20°C.

##### Biotin Solution:

##### Composition per 10.0mL:

Biotin.....	0.01mg
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**Preparation of Biotin Solution:** Add biotin to 10.0mL of absolute ethanol. Mix thoroughly.

**Preparation of Medium:** Add asolectin to 500.0mL of distilled/deionized water. Gently heat to 80°C. Mix thoroughly. Add other components, except artificial seawater and vitamin solution, to distilled/deionized water and bring volume to 831.0mL. Mix thoroughly. Adjust pH to 7.2. Au-

toclave for 15 min at 15 psi pressure–121°C. Aseptically add 167.0mL of sterile artificial seawater and 2.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Potomacoccus pottsi*.

#### PPB, Modified Caldwell and Bryant

##### Composition per liter:

Pancreatic digest of casein.....	2.0g
Yeast extract.....	2.0g
Cellobiose.....	1.0g
Glucose.....	1.0g
Maltose.....	1.0g
Starch.....	1.0g
Resazurin.....	1.0mg
Rumen fluid, clarified.....	150.0mL
Mineral solution I.....	100.0mL
Mineral solution II.....	100.0mL
Na <sub>2</sub> CO <sub>3</sub> solution.....	50.0mL
Hemin solution.....	10.0mL
L-Cysteine-HCl solution.....	10.0mL
Volatile fatty acid mixture.....	3.1mL
pH 6.8 ± 0.2 at 25°C	

##### Mineral Solution I:

##### Composition per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
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**Preparation of Mineral Solution I:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

##### Mineral Solution II:

##### Composition per 100.0mL:

NaCl.....	0.4g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.09g
CaCl <sub>2</sub> .....	0.05g

**Preparation of Mineral Solution II:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

##### Na<sub>2</sub>CO<sub>3</sub> Solution:

##### Composition per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	8.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

##### Hemin Solution:

##### Composition per 100.0mL:

Hemin.....	1.0g
NaOH (1N solution).....	10.0mL

**Preparation of Hemin Solution:** Add components to 100.0mL of distilled/deionized water. Mix thoroughly.

##### L-Cysteine-HCl Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl.....	0.25g
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**Preparation of L-Cysteine-HCl Solution:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

##### Volatile Fatty Acid Mixture:

##### Composition per 31.0mL:

Acetic acid.....	17.0mL
Propionic acid.....	6.0mL

Butyric acid.....	4.0mL
DL- $\alpha$ -Methylbutyric acid.....	1.0mL
Isobutyric acid.....	1.0mL
Isovaleric acid.....	1.0mL
n-Valeric acid.....	1.0mL

**Preparation of Volatile Fatty Acid Mixture:** Combine components. Mix thoroughly. Store under 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 100% CO<sub>2</sub>. Add components, except L-cysteine-HCl solution and Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Adjust pH to 6.8 with 1N NaOH. Distribute anaerobically 9.3mL volumes into Hungate tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.2mL of sterile L-cysteine-HCl solution and 0.5mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution. Check that final pH is 6.8. (Note: if not properly gassed with 100% CO<sub>2</sub>, the medium pH can be as high as 9.5.)

**Use:** For the cultivation of *Anaerovibrio lipolytica*, *Bacteroides* species, *Butyrivibrio crossotus*, *Butyrivibrio fibrisolvens*, *Eubacterium cellulosolvens*, *Eubacterium ruminantium*, *Fibrobacter succinogenes*, *Lachnospira multiparus*, *Megasphaera elsdenii*, *Rhodococcus torques*, *Ruminobacter amylophilus*, *Ruminococcus albus*, *Ruminococcus bromii*, *Ruminococcus flavifaciens*, *Selenomonas ruminantium*, *Succinomonas amylolytica*, *Succinovibrio dextrinisolvens*, and *Veillonella parvula*.

#### PPES II Medium

**Composition per liter:**

Agar.....	15.0g
Polypeptone.....	2.0g
Yeast extract.....	1.0g
Papaic digest of soybean meal.....	1.0g
Proteose peptone No. 3.....	1.0g
Ferric phosphate, soluble.....	0.1g
Marine mud extract.....	100.0mL

pH 7.6  $\pm$  0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Erythrobacter longus*, *Haloferrax mediterranei*, and *Roseobacter denitrificans*.

#### PPGA Medium

**Composition per liter:**

Agar.....	18.0g
Glucose.....	5.0g
Peptone.....	5.0g
NaCl.....	3.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Potato decoction.....	1.0L

pH 7.0  $\pm$  0.2 at 25°C

**Potato Decoction:**

**Composition per liter:**

Potatoes.....	200.0g
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**Preparation of Potato Decoction:** Peel and dice potatoes. Add 1.0L of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 20 min. Filter through two layers of cheesecloth. Bring volume of filtrate to 1.0L with distilled/deionized water.

**Preparation of Medium:** Combine components. Gently heat and bring to boiling. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Burkholderia glumae* and *Burkholderia plantarii*.

#### Presence-Absence Broth (P-A Broth)

**Composition per liter:**

Pancreatic digest of casein.....	10.0g
Lactose.....	7.5g
Pancreatic digest of gelatin.....	5.0g
Beef extract.....	3.0g
NaCl.....	2.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.375g
KH <sub>2</sub> PO <sub>4</sub> .....	1.375g
Sodium lauryl sulfate.....	0.05g
Bromocresol Purple.....	8.5mg

pH 6.8  $\pm$  0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 333.0mL. Mix thoroughly. Distribute into screw-capped 250.0mL milk dilution bottles in 50.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the detection of coliform bacteria in water from treatment plants or distribution systems using the presence-absence coliform test.

#### Propionigenium maris Medium

**Composition per 1014.0mL:**

Solution A.....	940.0mL
Solution E (NaHCO <sub>3</sub> solution).....	50.0mL
Solution F (Substrate solution).....	10.0mL
Solution G (Na <sub>2</sub> S-9H <sub>2</sub> O solution).....	10.0mL
Solution B (Trace elements solution SL-10).....	2.0mL
Solution C (Seven vitamin solution).....	1.0mL
Solution D (Selenite-tungstate solution).....	1.0mL

pH 7.2–7.4 at 25°C

**Solution A:**

**Composition per 940.0mL:**

NaCl.....	1.0g
Yeast extract.....	0.5g
KCl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	0.5mg

**Preparation of Solution A:** Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Prepare and dispense under 100% N<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C (Seven Vitamin Solution):**

**Composition per liter:**

Pyridoxine·HCl .....	300.0mg
Nicotinic acid .....	200.0mg
Thiamine·HCl .....	200.0mg
Calcium pantothenate .....	100.0mg
Cyanocobalamin .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Solution C (Seven Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Solution D (Selenite-Tungstate Solution):**

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution D (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E (NaHCO<sub>3</sub> Solution):**

**Composition per 50.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution E (NaHCO<sub>3</sub> Solution):** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F (Substrate Solution):**

**Composition per 10.0mL:**

Disodium succinate .....	2.5g
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**Preparation of Solution F (Substrate Solution):** Add disodium succinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 940.0mL of sterile solution A, aseptically and anaerobically add 1.0mL of sterile solution B, 1.0mL of sterile solution C, 1.0mL of sterile so-

lution D, 50.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Propionigenium maris*.

### ***Propionivibrio/Acetivibrio/Formivibrio Medium***

**Composition per 1012.0mL:**

Solution A .....	950.0mL
Solution E .....	30.0mL
Solution D (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution G .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
Solution C (Selenite-tungstate solution) .....	1.0mL
pH 6.7 ± 0.2 at 25°C	

#### **Solution A:**

**Composition per 950.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
NH <sub>4</sub> Cl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### **Preparation of Solution B (Trace Elements Solution SL-10):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C (Selenite-Tungstate Solution):**

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

#### **Preparation of Solution C (Selenite-Tungstate Solution):**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution D (Vitamin Solution):**

**Composition per liter:**

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg

Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution D (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### **Solution E:**

**Composition** per 30.0mL:

NaHCO <sub>3</sub> .....	1.5g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Solution F:**

**Composition** per 10.0mL:

Disodium maleate .....	1.6g
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**Preparation of Solution F:** Add disodium maleate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution G:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.25g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine 950.0mL of sterile solution A with 1.0mL of sterile solution B, 1.0mL of sterile solution C, 10.0mL of sterile solution D, 30.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G, in that order. Mix thoroughly. Final pH should be 6.7–6.8. Aseptically and anaerobically distribute into sterile tubes or flasks under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Propionivibrio dicarboxylicus*.

### ***Propionivibrio/Acetivibrio/Formivibrio Medium***

**Composition** per 1012.0mL:

Solution A .....	950.0mL
Solution E .....	30.0mL
Solution D (Vitamin solution).....	10.0mL
Solution F .....	10.0mL
Solution G .....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
Solution C (Selenite-tungstate solution).....	1.0mL
pH 7.7–7.9 at 25°C	

#### **Solution A:**

**Composition** per 950.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	1.4g
NH <sub>4</sub> Cl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Yeast extract.....	50.0mg
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
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CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C (Selenite-Tungstate Solution):**

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution C (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution D (Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution D (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### **Solution E:**

**Composition** per 30.0mL:

NaHCO <sub>3</sub> .....	1.5g
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**Preparation of Solution E:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Solution F:**

**Composition** per 10.0mL:

Cinnamic acid.....	1.6g
NaOH (1 <i>N</i> solution).....	variable

**Preparation of Solution F:** Add cinnamic acid to distilled/deionized water and bring volume to 8.0mL. Mix thoroughly. Add sufficient quantity of 1*N* NaOH solution to bring pH to 7.8. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution G:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.25g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically under 100% N<sub>2</sub> combine 950.0mL of sterile solution A with 1.0mL of sterile solution B, 1.0mL of sterile solution C, 10.0mL of sterile solution D, 30.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G, in that order. Mix thoroughly. Final pH should be 7.7–7.9. If necessary, add about 15.0mL of sterile anaerobic 5% Na<sub>2</sub>CO<sub>3</sub> solution to 1.0L of medium to adjust pH. Aseptically and anaerobically distribute into sterile tubes or flasks under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Acetivibrio multivorans*.

### *Prototheca Isolation Agar* (PIM)

**Composition per liter:**

Agar .....	20.0g
Glucose .....	10.0g
Potassium hydrogen phthalate .....	10.0g
NaOH .....	0.9g
NH <sub>4</sub> Cl .....	0.3g
5-Fluorocytosine .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> .....	0.1g
Thiamine-HCl .....	0.001g

pH 5.1 ± 0.1 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.1. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Prototheca moriformis* and *Prototheca ulmea*.

### Provasoli Medium

**Composition per liter:**

NaCl .....	11.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.35g
Na <sub>2</sub> SO <sub>4</sub> .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.75g
Tris(hydroxymethyl)aminomethane .....	0.5g
KCl .....	0.35g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.05g

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Leucothrix* species from marine habitats.

### *Pseudoamycolata halophobica* Medium

**Composition per liter:**

Glucose .....	5.0g
Peptone .....	5.0g
Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudoamycolata halophobica*.

### *Pseudobutyrvibrio* Medium

**Composition per liter:**

Disodium succinate .....	5.0g
Yeast extract .....	5.0g
NaCl .....	0.45g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.45g
K <sub>2</sub> HPO <sub>4</sub> .....	0.225g
KH <sub>2</sub> PO <sub>4</sub> .....	0.225g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.09g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
Indigocarmine .....	5.0mg
Rumen fluid, clarified .....	400.0mL
Glucose solution .....	20.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 6.6–6.8 at 25°C

**Glucose Solution:**

**Composition per 20.0mL:**

D-Glucose .....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	6.4g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

L-Cysteine-HCl-H <sub>2</sub> O .....	0.3g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Preparation of Medium:** Add components, except glucose solution, L-cysteine-HCl-H<sub>2</sub>O solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile glucose solution, 10.0mL of sterile L-cysteine-HCl solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 10.0mL of sterile NaHCO<sub>3</sub> solution to each tube. Mix thoroughly.

**Use:** For the cultivation of *Pseudobutyrvibrio ruminis*.

***Pseudomonas aeruginosa* Agar  
(PA Agar)  
(m-PA Agar)  
(m-*Pseudomonas aeruginosa* Agar)**

**Composition per liter:**

Agar .....	15.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	6.8g
L-Lysine-HCl .....	5.0g
NaCl .....	5.0g
Xylose .....	2.5g
Yeast extract .....	2.0g
Lactose .....	1.25g
Sucrose .....	1.25g
Ferric ammonium citrate .....	0.8g
Sulfapyridine .....	0.176g
Cycloheximide .....	0.15g
Phenol Red .....	0.08g
Nalidixic acid .....	0.037g
Kanamycin .....	8.5mg

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except sulfapyridine, cycloheximide, nalidixic acid, and kanamycin, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°–60°C. Readjust pH to 7.1. Aseptically add the sulfapyridine, cycloheximide, nalidixic acid, and kanamycin. Mix thoroughly. Pour into 50mm × 12mm Petri dishes in 3.0mL volumes.

**Use:** For the cultivation and estimation of numbers of *Pseudomonas aeruginosa* in water by the membrane filter method.

***Pseudomonas* Agar F**

**Composition per liter:**

Proteose peptone No. 3 .....	20.0g
Agar .....	15.0g
Glycerol .....	10.0g
Pancreatic digest of casein .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.73g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and observation of fluorescein production in *Pseudomonas* species.

***Pseudomonas* Agar P**

**Composition per liter:**

Proteose peptone No. 3 .....	20.0g
Agar .....	15.0g
Glycerol .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.4g

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation, cultivation, and differentiation of *Pseudomonas aeruginosa* on the basis of pigment production.

***Pseudomonas* Basal Mineral Medium**

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	12.5g
KH <sub>2</sub> PO <sub>4</sub> .....	3.8g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Carbon source (0.8M solution) .....	100.0mL
Trace elements solution .....	5.0mL

pH 7.2 ± 0.2 at 25°C

**Trace Elements Solution:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.232g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.174g
FeSO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ·6H <sub>2</sub> O .....	0.116g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.096g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.022g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	8.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	8.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Carbon Source:**

**Composition per 100.0mL:**

Glucose .....	14.4g
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**Preparation of Carbon Source:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Other carbon sources may replace glucose. Prepare 0.8M carbon source solution.

**Preparation of Medium:** Add components, except carbon source, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile carbon source. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and differentiation of *Pseudomonas* species based on their ability to grow on different carbon sources.

***Pseudomonas bathycetes* Medium**

**Composition per liter:**

NaCl .....	24.0g
Proteose peptone .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
MgCl <sub>2</sub> .....	5.3g
Yeast extract .....	3.0g
KCl .....	0.7g

pH 7.2–7.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Alteromonas haloplanktis*, *Alteromonas nigrifaciens*, *Pseudomonas bathycetes*, and *Pseudomonas elongata*.

***Pseudomonas* CFC Agar**

**Composition per liter:**

Pancreatic digest of gelatin .....	16.0g
Agar .....	11.0g
Pancreatic digest of casein .....	10.0g



K <sub>2</sub> SO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.4g
CFC selective supplement .....	10.0mL
Glycerol .....	10.0mL

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

#### CFC Selective Supplement:

**Composition** per 10.0mL:

Cephaloridine.....	0.05g
Fucidin.....	0.01g
Cetrimide .....	0.01g

**Preparation of CFC Selective Supplement:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except CFC selective supplement, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile CFC selective supplement. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of *Pseudomonas* species.

#### *Pseudomonas chlorotidismutans* Medium (DSMZ Medium 944)

**Composition** per 1001mL:

Solution A .....	900.0mL
Solution B .....	50.0mL
Solution C .....	50.0mL
Vitamin solution.....	1.0mL

pH 9.0 ± 0.2 at 25°C

#### Solution A:

**Composition** per 900mL:

Na-acetate·3H <sub>2</sub> O.....	2.72g
NaClO <sub>3</sub> .....	1.06g
KH <sub>2</sub> PO <sub>4</sub> .....	0.41g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.53g
Resazurin.....	0.5mg
Selenite-tungstate solution.....	4.0mL
Trace elements solution SL-10 .....	1.0mL

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Selenite-Tungstate Solution

**Composition** per liter:

NaOH.....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Solution B:

**Composition** per 50mL:

CaCl <sub>2</sub> .....	0.11g
MgCl <sub>2</sub> .....	0.10g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Solution C:

NaHCO <sub>3</sub> .....	3.73g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
NH <sub>4</sub> HCO <sub>3</sub> .....	0.44g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Vitamin Solution:

**Composition** per liter:

Vitamin B <sub>12</sub> .....	50.0mg
Pantothenic acid.....	50.0mg
Riboflavin.....	50.0mg
Alpha-lipoic acid.....	50.0mg
p-Aminobenzoic acid.....	50.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	50.0mg
Nicotinic acid.....	25.0mg
Nicotine amide.....	25.0mg
Biotin.....	20.0mg
Folic acid.....	20.0mg
Pyridoxamine-HCl.....	10.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub> gas. Aseptically and anaerobically combine 900.0mL sterile solution A, 50.0mL sterile solution B, 50.0mL sterile solution C, and 1.0mL sterile vitamin solution. Mix thoroughly. The pH should be 9.0. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Pseudomonas chlorotidismutans* (*Pseudomonas stutzeri*).

#### *Pseudomonas* CN Agar

**Composition** per liter:

Pancreatic digest of gelatin.....	16.0g
Agar.....	11.0g
Pancreatic digest of casein.....	10.0g
K <sub>2</sub> SO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.4g
CN selective supplement.....	10.0mL
Glycerol.....	10.0mL

#### CN Selective Supplement:

Cetrimide.....	0.1g
Sodium nalidixate.....	7.5mg

pH 7.1 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components, except CN selective supplement, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile CN selective supplement. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation and cultivation of *Pseudomonas* species.

### *Pseudomonas denitrificans* Medium (LMG 153)

**Composition per liter:**

Agar .....	15.0g
Glucose .....	10.0g
Yeast extract .....	5.0g
FeCl <sub>3</sub> solution .....	20.0mL

#### **FeCl<sub>3</sub> Solution:**

**Composition per 100.0mL:**

FeCl <sub>3</sub> .....	0.03g
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except FeCl<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 20.0mL of sterile FeCl<sub>3</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

### *Pseudomonas* Denitrification Medium

**Composition per liter:**

Glycerol .....	10.0g
KNO <sub>3</sub> .....	10.0g
Yeast extract .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.5g
Agar .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.8g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> .....	0.1g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and differentiation of *Pseudomonas* species based on their ability to produce pyocin and other fluorescent pigments during denitrification.

### *Pseudomonas halophila* Medium

**Composition per liter:**

Solution A .....	890.0mL
Solution B .....	100.0mL
Vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

#### **Solution A:**

**Composition per 890.0mL:**

NaCl .....	46.8g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	39.4g
Glycerol .....	5.0g

NH <sub>4</sub> Cl .....	1.0g
Trace elements solution SL-10 .....	1.0mL

#### **Trace Elements Solution SL-10:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Solution B:**

**Composition per 100.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
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**Preparation of Solution B:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### **Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Calcium pantothenate .....	5.0mg
Nicotinic acid .....	5.0mg
Robiflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
<i>p</i> -Aminobenzoic acid .....	1.0mg
Cyanocobalamin .....	0.01mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 890.0mL of cooled sterile solution A, 100.0mL of cooled sterile solution B, and 10.0mL of sterile vitamin solution. Mix thoroughly. Adjust pH to 7.0 with sterile 6N NaOH or HCl solutions.

**Use:** For the cultivation and maintenance of *Pseudomonas halophila*.

### *Pseudomonas* Isolation Agar

**Composition per liter:**

Peptone .....	20.0g
Agar .....	13.6g
K <sub>2</sub> SO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.4g
Irgasan (triclosan) .....	0.025g
Glycerol .....	20.0mL

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Pseudomonas* species.

### *Pseudomonas* Medium (ATCC Medium 59)

**Composition** per liter:

K <sub>2</sub> HPO <sub>4</sub> .....	1.15g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
Yeast extract.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.625g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.02g
Pyrrolidine.....	4.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add pyrrolidine to approximately 500.0mL of distilled/deionized water. Mix thoroughly. Adjust pH to 7.0. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Pseudomonas fluorescens*.

### *Pseudomonas* Medium (ATCC Medium 609)

**Composition** per liter:

Agar.....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	8.71g
Nitritotriacetic acid.....	1.91g
Na <sub>2</sub> SO <sub>4</sub> .....	0.57g
MgSO <sub>4</sub> .....	0.25g
FeSO <sub>4</sub> .....	0.5mg
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.5mg

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add nitritotriacetic acid to approximately 500.0mL of distilled/deionized water. Mix thoroughly. Adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

### *Pseudomonas* Medium A

**Composition** per liter:

Peptone.....	20.0g
Agar.....	15.0g
Glycerol.....	10.0g
K <sub>2</sub> SO <sub>4</sub> .....	10.0g
MgCl <sub>2</sub> .....	1.4g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 10 min at 10 psi pressure–115°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and production of pyocyanin by *Pseudomonas* species.

### *Pseudomonas* Medium B

**Composition** per liter:

Peptone.....	20.0g
Agar.....	15.0g

Glycerol.....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.5g
K <sub>2</sub> HPO <sub>4</sub> solution.....	100.0mL

pH 7.2 ± 0.2 at 25°C

### K<sub>2</sub>HPO<sub>4</sub> Solution:

**Composition** per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add components, except K<sub>2</sub>HPO<sub>4</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile K<sub>2</sub>HPO<sub>4</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and observation of fluoresein production by *Pseudomonas* species.

### *Pseudomonas* Medium No. 2

**Composition** per liter:

Agar.....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	6.0g
Succinic acid.....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.4g
NH <sub>4</sub> Cl.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.01g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.01g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Pseudomonas* species and *Psychrobacter immobilis*.

### *Pseudomonas pickettii* Medium

**Composition** per liter:

Agar.....	15.0g
Peptone.....	5.0g
NaCl.....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	2.39g
Yeast extract.....	2.0g
Beef extract.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.45g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Burkholderia pickettii*.

### *Pseudomonas saccharophila* Medium

**Composition** per 1015.0mL:

Agar.....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	4.8g
KH <sub>2</sub> PO <sub>4</sub> .....	4.4g
NH <sub>4</sub> Cl.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g

Solution A .....	5.0mL
Solution B .....	10.0mL

**Solution A:****Composition per 100.0mL:**

Ferric ammonium citrate .....	1.0g
CaCl <sub>2</sub> .....	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution B:****Composition per 100.0mL:**

Sucrose .....	10.0g
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**Preparation of Solution B:** Add sucrose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except solution A and solution B, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile solution A and sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas saccharophila* and other *Pseudomonas* species.

***Pseudomonas solanacearum* Medium****Composition per liter:**

Agar .....	17.0g
Peptone .....	10.0g
Glucose .....	5.0g
Pancreatic digest of casein .....	1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas solanacearum*.

***Pseudomonas syngii* Medium****Composition per liter:**

Agar .....	15.0g
Acid casein hydrolysate .....	7.5g
Sucrose .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	250.0mg
K <sub>2</sub> HPO <sub>4</sub> .....	500.0mg
Ammonium ferric citrate solution .....	20.0mL

**Ammonium Ferric citrate Solution:****Composition per 20.0mL:**

Ammonium ferric citrate .....	0.25g
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**Preparation of Ammonium Ferric citrate Solution:** Add ammonium ferric citrate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ammonium ferric citrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile ammonium ferric citrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas syngii*.

***Pseudomonas syringae* Selective Medium****Composition per liter:**

Agar .....	15.0g
L-Proline .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.08g
KH <sub>2</sub> PO <sub>4</sub> .....	0.02g
MnSO <sub>4</sub> ·4H <sub>2</sub> O solution .....	10.0mL
pH 6.8 ± 0.2 at 25°C	

**MnSO<sub>4</sub>·4H<sub>2</sub>O Solution:****Composition per 10.0mL:**

MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	2.1g
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**Preparation of MnSO<sub>4</sub>·4H<sub>2</sub>O Solution:** Add 2.1g of MnSO<sub>4</sub>·4H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except MnSO<sub>4</sub>·4H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.8. Autoclave for 10 min at 10 psi pressure–115°C. Cool to 45°–50°C. Aseptically add sterile MnSO<sub>4</sub>·4H<sub>2</sub>O solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the selective isolation and cultivation of *Pseudomonas syringae*.

**PT Agar****Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	4.0g
Yeast extract .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

**PTYG Medium  
(LMG Medium 238)****Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
Tryptone .....	5.0g
Yeast extract .....	5.0g
Glucose .....	5.0g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Sphingobium herbicidovorans* and *Sphingomonas pruni*.

**PTYG Medium  
(DSMZ Medium 914)****Composition per liter:**

Glucose .....	10.0g
Peptone .....	5.0g
Tryptone .....	5.0g

Yeast extract .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.6g
CaCl <sub>2</sub> .....	0.06g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Kineococcus radiotolerans*.

### Purple Agar

#### Composition per liter:

Agar .....	15.0g
Proteose peptone No. 3 .....	10.0g
NaCl .....	5.0g
Beef extract .....	1.0g
Bromcresol Purple .....	0.02g
Carbohydrate solution .....	20.0mL
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

#### Carbohydrate Solution:

##### Composition per 20.0mL:

Carbohydrate .....	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 20.0mL. For expensive carbohydrates, 5.0g may be used instead of 10.0g. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 9.8mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 0.2mL of sterile carbohydrate solution to each tube. Mix thoroughly. Allow tubes to cool in a slanted position.

**Use:** For the preparation of carbohydrate media used in fermentation studies for the identification of bacteria, especially members of the Enterobacteriaceae. Bacteria that can ferment the carbohydrate turn the medium yellow.

### Purple Broth (Purple Carbohydrate Broth)

#### Composition per liter:

Proteose peptone No. 3 .....	10.0g
NaCl .....	5.0g
Beef extract .....	1.0g
Bromcresol Purple .....	0.015g
Carbohydrate solution .....	20.0mL
pH 6.8 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

#### Carbohydrate Solution:

##### Composition per 20.0mL:

Carbohydrate .....	10.0g
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**Preparation of Carbohydrate Solution:** Add carbohydrate to distilled/deionized water and bring volume to 20.0mL. For expensive carbohydrates, 5.0g may be used instead of 10.0g. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except carbohydrate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 9.8mL volumes. Auto-

clave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 0.2mL of sterile carbohydrate solution to each tube. Mix thoroughly.

**Use:** For the preparation of carbohydrate media used in fermentation studies for the identification of bacteria, especially members of the Enterobacteriaceae. Bacteria that can ferment the carbohydrate turn the medium yellow.

### Purple Lactose Agar

#### Composition per liter:

Agar .....	10.0g
Lactose .....	10.0g
Peptone .....	5.0g
Beef extract .....	3.0g
Bromcresol Purple .....	0.025g
pH 6.8 ± 0.1 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Allow tubes to cool in a slanted position.

**Use:** For the detection and differentiation of members of the Enterobacteriaceae. Bacteria that can ferment lactose turn the medium yellow.

### PW Medium (LMG 182)

#### Composition per 1100.0mL:

Agar .....	12.0g
Papaic digest of soybean meal .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.2g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Trypticase peptone .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
Glutamine solution .....	50.0mL
Bovine serum albumin solution .....	30.0mL
Phenol Red (0.2% solution) .....	10.0mL
Solution A .....	10.0mL

#### Glutamine Solution:

##### Composition per 50.0mL:

L-Glutamine .....	4.0g
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**Preparation of Glutamine Solution:** Add glutamine to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

#### Bovine Serum Albumin Solution:

##### Composition per 50.0mL:

Bovine serum albumin, fraction V .....	10.0g
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**Preparation of Bovine Serum Albumin Solution:** Add bovine serum albumin to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

#### Solution A:

##### Composition per 101.0mL:

NaOH (0.05N solution) .....	100.0mL
Hemin chloride .....	0.1g

**Preparation of Medium:** Add components, except glutamine solution and bovine serum albumin solution, to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile glutamine solution

and 10.0mL of sterile bovine serum albumin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Xylella fastidiosa*.

**PY CMC Medium**  
(Peptone Yeast Extract  
Carboxymethyl Cellulose Medium)

**Composition per liter:**

Agar .....	15.0g
Carboxymethyl cellulose .....	10.0g
NaCl .....	5.0g
Polypeptone .....	5.0g
Yeast extract .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
Na <sub>2</sub> CO <sub>3</sub> solution .....	100.0mL

pH 9.5 ± 0.2 at 25°C

**Na<sub>2</sub>CO<sub>3</sub> Solution:**

**Composition per 100.0mL:**

Na <sub>2</sub> CO <sub>3</sub> .....	10.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 9.5 if necessary. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of alkalophilic *Bacillus* species.

**PYb Agar**

**Composition per liter:**

Agar .....	20.0g
Proteose peptone .....	1.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> solution .....	32.0mL
Na <sub>2</sub> HPO <sub>4</sub> solution .....	8.0mL
CaCl <sub>2</sub> solution .....	4.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	2.5mL

pH 6.5 ± 0.5 at 25°C

**CaCl<sub>2</sub> Solution:**

**Composition per 100.0mL:**

CaCl <sub>2</sub> .....	0.75g
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**Preparation of CaCl<sub>2</sub> Solution:** Add CaCl<sub>2</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:**

**Composition per 100.0mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.8g
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**Preparation of MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 9.8g of MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Na<sub>2</sub>HPO<sub>4</sub> Solution:**

**Composition per 100.0mL:**

Na <sub>2</sub> HPO <sub>4</sub> .....	6.7g
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**Preparation of Na<sub>2</sub>HPO<sub>4</sub> Solution:** Add Na<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**KH<sub>2</sub>PO<sub>4</sub> Solution:**

**Composition per 100.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	3.4g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Add components, except KH<sub>2</sub>PO<sub>4</sub> solution, Na<sub>2</sub>HPO<sub>4</sub> solution, CaCl<sub>2</sub> solution, and MgSO<sub>4</sub>·7H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 953.5mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 32.0mL of sterile KH<sub>2</sub>PO<sub>4</sub> solution, 8.0mL of sterile Na<sub>2</sub>HPO<sub>4</sub> solution, 4.0mL of sterile CaCl<sub>2</sub> solution, and 2.5mL of sterile MgSO<sub>4</sub>·7H<sub>2</sub>O solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Acanthamoeba hatchetti*, *Acanthamoeba jacobsoni*, *Acanthamoeba polyphaga*, *Echinamoeba exundans*, *Naegleria gruberi*, *Paratetramitus jugosus*, *Rhizamoeba* species, *Tetramitus rostratus*, and *Vahlkampffia lobospinosa*.

**PYCS Medium**

**Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	45.0mg
Fructose solution .....	50.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
Wolfe's mineral solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Fructose Solution:**

**Composition per 50.0mL:**

D-Fructose .....	5.0g
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**Preparation of Fructose Solution:** Add D-fructose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.68g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Wolfe's Mineral Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrotriacetic acid to approximately 500.0mL of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0L with remaining water and add remaining components one at a time.

**Preparation of Medium:** Add components, except fructose solution and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 50.0mL of sterile fructose solution and 10.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Adjust pH to 7.2. Aseptically distribute into sterile tubes or flasks. Fill containers to capacity.

**Use:** For the cultivation of *Rhodospirillum rubrum* and *Thiobacillus thiooxidans*.

### PYE Medium

**Composition per liter:**

Yeast extract .....	4.0g
Sodium pyruvate .....	2.2g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
Trace elements solution SL-6 .....	1.0mL

### Trace Elements Solution SL-6:

**Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Heliobacterium modestocaldum*.

### PYEA Agar

**Composition per liter:**

Agar .....	15.0g
Peptone .....	10.0g
Yeast extract .....	10.0g
NaCl .....	5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Blastobacter natatorius* and *Deinobacter grandis*.

### PYG Agar

**Composition per liter:**

Agar .....	20.0g
Proteose peptone .....	20.0g
Yeast extract .....	1.0g

Glucose solution .....	50.0mL
Sodium citrate solution .....	34.0mL
Ferric ammonium sulfate .....	10.0mL
KH <sub>2</sub> PO <sub>4</sub> solution .....	10.0mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> HPO <sub>4</sub> solution .....	10.0mL
CaCl <sub>2</sub> solution .....	8.0mL

pH 6.5 ± 0.5 at 25°C

### Glucose Solution:

**Composition per 100.0mL:**

Glucose .....	36.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 55°C.

### MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:

**Composition per 100.0mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	9.8g
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**Preparation of MgSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 9.8g of MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### Ferric Ammonium Sulfate Solution:

**Composition per 100.0mL:**

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.135g
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**Preparation of Ferric Ammonium Sulfate Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### Na<sub>2</sub>HPO<sub>4</sub> Solution:

**Composition per 100.0mL:**

Na <sub>2</sub> HPO <sub>4</sub> .....	6.7g
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**Preparation of Na<sub>2</sub>HPO<sub>4</sub> Solution:** Add Na<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### Sodium Citrate Solution:

**Composition per 100.0mL:**

Sodium citrate·2H <sub>2</sub> O .....	2.9g
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**Preparation of Sodium Citrate Solution:** Add sodium citrate·2H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### KH<sub>2</sub>PO<sub>4</sub> Solution:

**Composition per 100.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	3.4g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### CaCl<sub>2</sub> Solution:

**Composition per 100.0mL:**

CaCl <sub>2</sub> .....	0.75g
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**Preparation of CaCl<sub>2</sub> Solution:** Add CaCl<sub>2</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 6.5. Autoclave for 25 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Add components, except glucose solution, sodium citrate solution, ferric ammonium sulfate solution, KH<sub>2</sub>PO<sub>4</sub> solution, Na<sub>2</sub>HPO<sub>4</sub> solution,

CaCl<sub>2</sub> solution, and MgSO<sub>4</sub>·7H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 868.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 50.0mL of sterile glucose solution, 34.0mL of sterile sodium citrate solution, 10.0mL of sterile ferric ammonium sulfate solution, 10.0mL of sterile KH<sub>2</sub>PO<sub>4</sub> solution, 10.0mL of sterile Na<sub>2</sub>HPO<sub>4</sub> solution, 8.0mL of sterile CaCl<sub>2</sub> solution, and 10.0mL of sterile MgSO<sub>4</sub>·7H<sub>2</sub>O solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Acanthamoeba* spp.

**PYG Medium for *Spirillum*  
(Peptone Yeast Extract Glucose  
Medium for *Spirillum*)**

**Composition per liter:**

Agar .....	15.0g
Peptone.....	10.0g
Yeast extract.....	5.0g
Glucose .....	3.0g

pH 7.2 ± 0.2 at 25°C

**Glucose Solution:**

**Composition per 10.0mL:**

D-Glucose.....	3.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Spirillum pleomorphicum*.

**PYGS Agar  
(Peptone Yeast Glucose Seawater Agar)  
(ATCC Medium 1973)**

**Composition per liter:**

Agar .....	15.0g
Glucose.....	3.0g
Peptone.....	1.25g
Yeast extract.....	1.25g
Seawater.....	25.0mL

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to cold distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of marine bacteria.

**PYGV Agar**

**Composition per liter:**

Agar .....	15.0g
Peptone.....	0.25g
Yeast extract.....	0.25g
Hutner's basal salts solution .....	20.0mL
Glucose solution .....	10.0mL
Vitamin solution 2×.....	5.0mL

pH 7.5 ± 0.2 at 25°C

**Hutner's Basal Salts Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	29.7g
Nitrilotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>7</sub> ·O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44".....	50.0mL

**"Metals 44":**

**Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Glucose Solution:**

**Composition per 10.0mL:**

D-Glucose.....	0.25g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Vitamin Solution 2×:**

**Composition per liter:**

Pyridoxine-HCl.....	20.0mg
p-Aminobenzoic acid .....	10.0mg
Calcium DL-pantothenate.....	10.0mg
Nicotinamide .....	10.0mg
Riboflavin.....	10.0mg
Thiamine-HCl.....	10.0mg
Biotin.....	4.0mg
Folic acid .....	4.0mg
Vitamin B <sub>12</sub> .....	0.2mg

**Preparation of Vitamin Solution 2×:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Store in the dark at 5°C.

**Preparation of Medium:** Add components, except glucose solution and vitamin solution 2×, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.5 with 6N KOH (approximately 6 drops). Autoclave for 20 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile glucose solution and 5.0mL of sterile vitamin solution 2×. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Blastobacter denitrificans*, *Planctomyces limnophilus*, and *Gemmobacter aquatilis*.

**PYGV Marine Medium  
(Peptone Yeast Extract Glucose  
Vitamin Marine Medium)**

**Composition per liter:**

Agar.....	15.0g
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Peptone.....	0.25g
Yeast extract.....	0.25g
Mineral salt solution .....	20.0mL
Glucose solution .....	10.0mL
Vitamin solution.....	5.0mL

pH 7.5 ± 0.2 at 25°C

**Mineral Salt Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	29.7g
Nitrilotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.099g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.013g
“Metals 44”.....	50.0mL

**Preparation of Mineral Salt Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Readjust pH to 7.2.

**“Metals 44”:****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	0.018g

**Preparation of “Metals 44”:** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Glucose Solution:****Composition per 100.0mL:**

D-Glucose.....	2.5g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl.....	0.02g
<i>p</i> -Aminobenzoic acid.....	0.01g
Calcium D-pantothenate.....	0.01g
Nicotinamide.....	0.01g
Riboflavin .....	0.01g
Thiamine·HCl .....	0.01g
Biotin .....	4.0mg
Folic acid.....	4.0mg
Cyanocobalamin .....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and vitamin solution, to seawater and bring volume to 985.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile glucose solution and 5.0mL of sterile vitamin solution. Mix thoroughly. Adjust pH to 7.5 with sterile KOH if necessary. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Planctomyces brasiliensis*.

**PYGV Medium**  
**(Peptone Yeast Extract**  
**Glucose Vitamin Medium)**

**Composition per liter:**

Agar.....	15.0g
Peptone.....	0.25g
Yeast extract .....	0.25g
Mineral salt solution.....	20.0mL
Glucose solution.....	10.0mL
Vitamin solution.....	5.0mL

pH 7.5 ± 0.2 at 25°C

**Mineral Salt Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	29.7g
Nitrilotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	99.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	12.67mg
“Metals 44”.....	50.0mL

**Preparation of Mineral Salt Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Readjust pH to 7.2.

**“Metals 44”:****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	0.018g

**Preparation of “Metals 44”:** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Glucose Solution:****Composition per 100.0mL:**

D-Glucose.....	2.5g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl.....	0.02g
<i>p</i> -Aminobenzoic acid.....	0.01g
Calcium D-pantothenate.....	0.01g
Nicotinamide.....	0.01g
Riboflavin.....	0.01g
Thiamine·HCl.....	0.01g
Biotin.....	4.0mg
Folic acid.....	4.0mg
Cyanocobalamin.....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and vitamin solution, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile glucose solution and 5.0mL of sterile vitamin solution. Mix thoroughly. Adjust pH to 7.5 with ster-

ile KOH if necessary. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Blastobacter aggregatus*, *Blastobacter capsulatus*, *Blastobacter denitrificans*, and *Planctomyces limnophilus*.

#### PYGV Medium

##### Composition per liter:

Agar .....	15.0g
Peptone.....	0.25g
Yeast extract.....	0.25g
Mineral solution.....	20.0mL
Glucose solution.....	10.0mL
Vitamin solution.....	5.0mL

pH 7.5 ± 0.2 at 25°C

##### Mineral Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	29.7g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	12.67g
Nitrilotriacetic acid.....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
"Metals 44" solution.....	50.0mL

**Preparation of Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L. Store at 5°C.

##### "Metals 44":

##### Composition per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
EDTA.....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	0.018g

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

##### Glucose Solution:

##### Composition per 100.0mL:

D-Glucose.....	2.5g
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**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

##### Vitamin Solution:

##### Composition per liter:

Pyridoxin-HCl.....	0.02g
p-Aminobenzoic acid.....	0.01g
Ca-pantothenate.....	0.01g
Nicotinamide.....	0.01g
Riboflavin.....	0.01g
Thiamine-HCl.....	0.01g
Biotin.....	4.0mg
Folic acid.....	4.0mg
Vitamin B <sub>12</sub> .....	0.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L.

**Preparation of Medium:** Add components, except glucose solution and vitamin solution, to distilled/deionized water and bring volume to 985.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 20 min at 15 psi

pressure—121°C. Cool to 60°C. Aseptically add 10.0mL of sterile glucose solution and 5.0mL of sterile vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the enrichment of *Stella* species from polluted waters.

#### Pyridine Medium

##### Composition per 1001.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.61g
KH <sub>2</sub> PO <sub>4</sub> .....	0.39g
KCl.....	0.25g
Yeast extract.....	0.15g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.13g
Pyridine.....	1.0mL
Trace elements solution.....	1.0mL

##### Trace Elements Solution:

##### Composition per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	40.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	40.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	20.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	5.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	4.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.4mg
NaCl.....	1.0g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except pyridine, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure—121°C. Cool to room temperature. In a fume hood, aseptically add 1.0mL of pyridine. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Use polyurethane foam closures to eliminate odors caused by volatilization of pyridine.

**Use:** For the cultivation of *Micrococcus luteus*.

#### Pyrobaculum Medium

##### Composition per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Peptone.....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
Yeast extract.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Resazurin.....	1.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

**Preparation of Medium:** Add components, except peptone, yeast extract, and Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 6.0 using 8N NaOH. Sparge with 100% N<sub>2</sub> for 30 min. Add peptone, yeast extract, and Na<sub>2</sub>S·9H<sub>2</sub>O. Bring pH back to 6.0 using 10N H<sub>2</sub>SO<sub>4</sub>. Anaerobically distribute into sterile tubes or flasks under 100% N<sub>2</sub>. Do not autoclave medium.

If not used immediately, heat the medium to 90°C for 60 min on each of 3 consecutive days.

**Use:** For the cultivation and maintenance of *Pyrobaculum islandicum*.

#### *Pyrococcus endeavori* Medium ES4

**Composition** per 3.0L:

Solution A	1.0L
Solution B	1.0L
Solution C	1.0L

#### **Solution A:**

**Composition** per liter:

NaCl	47.8g
Na <sub>2</sub> SO <sub>4</sub>	8.0g
KCl	1.4g
NaHCO <sub>3</sub>	0.4g
KBr	0.2g
H <sub>3</sub> BO <sub>3</sub>	0.06g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B:**

**Composition** per liter:

MgCl <sub>2</sub> ·6H <sub>2</sub> O	21.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	3.0g
SrCl <sub>2</sub> ·6H <sub>2</sub> O	0.05g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

**Composition** per liter:

Sodium acetate	50.0g
NH <sub>4</sub> Cl	12.5g
K <sub>2</sub> HPO <sub>4</sub>	7.0g

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 1.0L of sterile solution A with 1.0L of sterile solution B and 1.0L of sterile solution C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Pyrococcus endeavori*.

#### *Pyrococcus furiosus* Medium

**Composition** per liter:

NaCl	13.8g
Pancreatic digest of casein	5.0g
Yeast extract	5.0g
Maltose	5.0g
MgSO <sub>4</sub>	3.5g
MgCl <sub>2</sub>	2.75g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
CaCl <sub>2</sub>	0.75g
KCl	0.325g
NaBr	50.0mg
KI	50.0mg
H <sub>3</sub> BO <sub>3</sub>	15.0mg
SrCl <sub>2</sub>	7.5mg
Citric acid	5.0mg
Resazurin	2.5mg
Mineral solution	10.0mL

pH 6.8 ± 0.2 at 25°C

#### **Mineral Solution:**

**Composition** per liter:

Nitriloacetic acid	1.0g
MnSO <sub>4</sub>	0.5g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	1.1g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	0.3g
EDTA	0.292g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Mix thoroughly. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of high cell concentrations of *Pyrococcus furiosus*.

#### *Pyrococcus* Medium

**Composition** per liter:

Sulfur	30.0g
NaCl	13.85g
Peptone	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
Yeast extract	1.0g
CaCl <sub>2</sub>	0.75g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
KCl	0.325g
NaBr	0.05g
H <sub>3</sub> BO <sub>3</sub>	15.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O	7.5mg
Citric acid	5.0mg
(NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> ) <sub>2</sub>	2.0mg
Resazurin	1.0mg
KI	0.05mg
Trace minerals solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL

#### **Trace Minerals Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·xH <sub>2</sub> O	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
CoSO <sub>4</sub> (or CoCl <sub>2</sub> )	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
ZnSO <sub>4</sub>	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub>	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoSO <sub>4</sub> 2H <sub>2</sub> O	0.01g

**Preparation of Trace Minerals Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5 with H<sub>2</sub>SO<sub>4</sub>. Do not autoclave. Sterilize by steaming at 100°C for 30 min on 3 consecutive days. Before inoculation, add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Pyrococcus furiosus* and *Pyrococcus woesei*.

#### *Pyrococcus/Staphylothermus* Medium

**Composition per 1010.0mL:**

Sulfur, powdered	30.0g
NaCl	13.85g
Peptone	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	2.0g
Yeast extract	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.75g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
KCl	0.325g
NaBr	0.05g
H <sub>3</sub> BO <sub>3</sub>	0.015g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O	7.5mg
Citric acid	5.0mg
Resazurin	1.0mg
KI	0.05mg
Trace elements solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL

pH 6.5 ± 0.2 at 25°C

#### **Trace Elements Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L.

Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Bring pH to 6.5 using 10N H<sub>2</sub>SO<sub>4</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Immediately prior to inoculation, add 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each 10.0mL of medium. Check that final pH is 6.5.

**Use:** For the cultivation and maintenance of *Pyrococcus furiosus*, *Pyrococcus woesei*, and *Staphylothermus marinus*.

#### *Pyrodictium abyssi* Medium

**Composition per liter:**

Sulfur, powdered	30.0g
NaCl	13.85g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.75g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
Yeast extract	0.5g
KCl	0.325g
NaBr	0.05g
H <sub>3</sub> BO <sub>3</sub>	0.015g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O	7.5mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	2.0mg
Resazurin	1.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	0.1mg
KI	0.05mg
Trace elements solution	10.0mL

pH 5.5–6.0 at 25°C

#### **Trace Elements Solution:**

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Bring pH to 5.5 using 10N H<sub>2</sub>SO<sub>4</sub>. Anaerobically distribute into tubes or flasks making sure to evenly distribute sulfur. Do not autoclave. For immediate use, heat the medium in a boiling water bath for 60 min prior to inoculation. For storage of medium, heat medium in a boiling water bath for 60 min on 3 consecutive days. Store at room temperature.

**Use:** For the cultivation and maintenance of *Pyrodictium abyssi*.

**Pyrodictium Medium****Composition** per liter:

Sulfur, powdered	30.0g
NaCl	13.85g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
Yeast extract	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.75g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
KCl	0.325g
NaBr	0.05g
H <sub>3</sub> BO <sub>3</sub>	0.015g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O	7.5mg
Citric acid	5.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	2.0mg
Resazurin	1.0mg
KI	0.05mg
Trace elements solution	10.0mL

pH 5.5 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Adjust pH to 7.0. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Bring pH to 5.5 using 10N H<sub>2</sub>SO<sub>4</sub>. Anaerobically distribute into tubes or flasks, making sure to evenly distribute sulfur. Do not autoclave. For immediate use, heat the medium in a boiling water bath for 60 min prior to inoculation. For storage of medium, heat medium in a boiling water bath for 60 min on 3 consecutive days. Store at room temperature.

**Use:** For the cultivation and maintenance of *Pyrodictium brockii*, *Pyrodictium occultum*, and a consortium consisting of *Lactobacillus brevis*, *Streptococcus lactis*, and *Saccharomyces cerevisiae*.

**Pyrolobus fumarii Medium  
(DSMZ Medium 792)****Composition** per liter:

NaCl	13.850g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.75g
KNO <sub>3</sub>	1.0g

KH <sub>2</sub> PO <sub>4</sub>	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.375g
KCl	0.325g
NaBr	0.05g
H <sub>3</sub> BO <sub>3</sub>	0.015g
SrCl <sub>2</sub> ·6H <sub>2</sub> O	7.5mg
Resazurin	1.0mg
Trace elements solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
KI solution	0.05mL

pH 5.5 ± 0.2 at 25°C

**Trace Elements Solution:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.30mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 1.0 with H<sub>2</sub>SO<sub>4</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**KI Solution:****Composition** per 10mL:

KI	5.0mg
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**Preparation of KI Solution:** Add KI to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into serum bottles under 80% H<sub>2</sub> + 20% CO<sub>2</sub>, e.g., 20mL into 120mL serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically inject Na<sub>2</sub>S·9H<sub>2</sub>O solution, 0.2mL per 20mL medium. Mix thoroughly. Adjust pH to 5.5. After inoculation pressurize vials to 2 bar overpressure with 0% H<sub>2</sub> + 20% CO<sub>2</sub> gas mixture.

**Use:** For the cultivation of *Pyrolobus fumarii*.

**Pyrrolidone Agar****Composition** per liter:

Noble agar	21.0g
K <sub>2</sub> HPO <sub>4</sub>	5.65g
KH <sub>2</sub> PO <sub>4</sub>	2.95g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.0g
Pyrrolidone carboxylic acid solution	30.0mL

NaOH solution .....	30.0mL
Trace metals .....	6.3mL

### Pyrrolidone Carboxylic Acid Solution:

<b>Composition</b> per 300.0mL:	
Pyrrolidone carboxylic acid .....	50.0g

**Preparation of Pyrrolidone Carboxylic Acid Solution:** Add pyrrolidone carboxylic acid to distilled/deionized water and bring volume to 300.0mL. Mix thoroughly. Filter sterilize.

### NaOH Solution:

<b>Composition</b> per 100.0mL:	
NaOH .....	5.0g

**Preparation of Resazurin Solution:** Add NaOH to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

### Trace Metals:

<b>Composition</b> per 100.0mL:	
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.13g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.02g

**Preparation of Trace Metals:** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except pyrrolidone carboxylic acid solution and NaOH solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 30.0mL of sterile pyrrolidone carboxylic acid solution and 30.0mL of sterile NaOH solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas fluorescens*.

### Quinoline Medium

<b>Composition</b> per 1000.2mL:	
K <sub>2</sub> HPO <sub>4</sub> .....	0.61g
KH <sub>2</sub> PO <sub>4</sub> .....	0.39g
KCl .....	0.25g
Yeast extract .....	0.1g
Wolfe's mineral solution .....	10.0mL
Quinoline .....	0.2mL

### Wolfe's Mineral Solution:

<b>Composition</b> per liter:	
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water.

Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Add components, except quinoline, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. In a fume hood, aseptically add 0.2mL of quinoline. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Use polyurethane foam closures to eliminate odors caused by volatilization of quinoline.

**Use:** For the cultivation of *Rhodococcus* species.

### Quinolinic Acid Medium

#### Composition per liter:

Quinolinic acid .....	1.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.1g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g

**Preparation:** Add quinolinic acid to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Bring pH to 7.0 with NaOH. Add other components. Bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of microorganisms that can utilize quinolinic acid as sole carbon source.

### R Agar with 3% NaCl

#### Composition per liter:

NaCl .....	30.0g
Agar .....	20.0g
Peptone .....	10.0g
Casamino acids .....	5.0g
Malt extract .....	5.0g
Yeast extract .....	5.0g
Beef extract .....	2.0g
Glycerol .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Tween 80 .....	50.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Rhodococcus marinonascens*.

### R Agar with 5% NaCl

#### Composition per liter:

NaCl .....	50.0g
Agar .....	20.0g
Peptone .....	10.0g
Casamino acids .....	5.0g
Malt extract .....	5.0g
Yeast extract .....	5.0g
Beef extract .....	2.0g
Glycerol .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Tween 80 .....	50.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Marinococcus albus*, *Marinococcus halophilus*, and other *Marinococcus* species.

### R8 Medium (DSMZ Medium 912)

**Composition** per liter:

NaHCO <sub>3</sub> .....	2.52g
MOPS.....	2.1g
NaCl.....	1.0g
Glucose.....	0.9g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.6g
Cysteine-HCl.....	0.4g
NH <sub>4</sub> Cl.....	0.3g
KCl.....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
Yeast extract.....	0.19g
Peptone.....	0.19g
Na-Pantothenate.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.015g
Resazurin.....	0.5mg
Rumen fluid.....	50.0mL
Na-Pantothenate solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL

pH 7.2 ± 0.2 at 25°C

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

### Rumen Fluid:

**Composition** per 50.0mL:

Rumen fluid, clarified.....	50.0mL
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**Preparation of Rumen Fluid:** Sparge clarified rumen fluid with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Na-Pantothenate Solution:

**Composition** per 10.0mL:

Na-Pantothenate.....	0.1g
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**Preparation of Na-Pantothenate Solution:** Add Na-pantothenate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub>, pantothenate solution, Na<sub>2</sub>S·9H<sub>2</sub>O, cysteine-HCl, and rumen fluid, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to room tempera-

ture while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add solid bicarbonate, sodium sulfide and cysteine-HCl. Adjust pH to 7.2. Distribute under 80% N<sub>2</sub> + 20% CO<sub>2</sub> atmosphere into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium, 50.0mL rumen fluid and 10.0mL Na-pantothenate solution. Mix thoroughly. The final pH of the medium should be 7.2.

**Use:** For the cultivation of *Spirochaeta* spp.

### R2A Agar

**Composition** per liter:

Agar.....	15.0g
Yeast extract.....	0.5g
Acid hydrolysate of casein.....	0.5g
Glucose.....	0.5g
Soluble starch.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
Sodium pyruvate.....	0.3g
Pancreatic digest of casein.....	0.25g
Peptic digest of animal tissue.....	0.25g
MgSO <sub>4</sub> , anhydrous.....	0.024g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat with mixing and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Do not overheat. Pour into sterile Petri dishes or leave in tubes.

**Use:** For use in standard methods for pour plate, spread plate, and membrane filter analysis to enumerate heterotrophic bacteria from potable waters.

### R2A Agar, Modified

**Composition** per liter:

Agar.....	15.0g
NH <sub>4</sub> Cl.....	0.8g
KNO <sub>3</sub> .....	0.505g
Casamino acids.....	0.5g
Glucose.....	0.5g
Peptone.....	0.5g
Sodium pyruvate.....	0.5g
Starch, soluble.....	0.5g
Yeast extract.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
KH <sub>2</sub> PO <sub>4</sub> .....	0.25g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	20.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	15.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	7.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	5.0mg
Na <sub>2</sub> SO <sub>4</sub> .....	5.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.5mg
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
NiSO <sub>4</sub> ·6H <sub>2</sub> O.....	0.5mg
ZnCl <sub>2</sub> .....	0.5mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.3mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	10.0µg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Azoarcus toluolyticus*.

### R3A Agar

#### Composition per liter:

Agar .....	15.0g
Yeast extract .....	1.0g
Acid hydrolysate of casein .....	1.0g
Glucose .....	1.0g
Soluble starch .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
Sodium pyruvate .....	0.6g
Pancreatic digest of casein .....	0.5g
Peptic digest of animal tissue .....	0.5g
MgSO <sub>4</sub> , anhydrous .....	0.048g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat with mixing and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Do not overheat. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of heterotrophic bacteria from potable waters.

### Rabbit Dung Agar

#### Composition per liter:

Rabbit dung .....	20.0g
Agar .....	15.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add rabbit dung to 1.0L of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 20 min. Filter through Whatman #1 filter paper. Bring volume of filtrate to 1.0L with distilled/deionized water. Add agar. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

### R8AH Medium (DSMZ Medium 651)

#### Composition per liter:

Malic acid .....	2.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.25g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
EDTA .....	0.02g
Ferric citrate .....	0.01g
Vitamin solution .....	7.5mL
Trace elements solution .....	1.0mL

pH 6.9 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per 100.0mL:

Ferric citrate .....	0.3g
EDTA .....	0.05g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	2.0mg
H <sub>3</sub> BO <sub>3</sub> .....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
ZnSO <sub>4</sub> .....	1.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Thiamine-HCl .....	0.4g
Nicotinic acid .....	0.2g
Nicotinamide .....	0.2g
Biotin .....	8.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add malic acid to approximately 500.0mL of distilled/deionized water. Adjust pH to 6.9 with NaOH. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Adjust pH to 6.9. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodocyclus tenuis*, *Rhodopseudomonas rutila*, *Rhodospirillum photometricum*, and *Rhodospirillum rubrum*.

### Raper *Achyla* Medium No. 1

#### Composition per liter:

Agar .....	20.0g
Lentil (hot water extract) .....	10.0g
Starch, soluble .....	3.0g
Peptone .....	1.0g
CaCl <sub>2</sub> .....	1.0μg
FeCl <sub>3</sub> .....	1.0μg
KH <sub>2</sub> PO <sub>4</sub> .....	1.0μg
MgSO <sub>4</sub> .....	1.0μg
ZnSO <sub>4</sub> .....	1.0μg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Achyla* species.

### Raper *Achyla* Medium No. 2

#### Composition per liter:

Agar .....	20.0g
Starch, soluble .....	3.0g
Inositol .....	1.0g
Peptone .....	1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Achyla* species.

### Rappaport-Vassiliadis Enrichment Broth (RV Enrichment Broth)

#### Composition per 1110.0mL:

NaCl .....	8.0g
Papaic digest of soybean meal .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.6g
Magnesium chloride solution .....	100.0mL
Malachite Green solution .....	10.0mL

pH 5.2 ± 0.2 at 25°C



**Source:** This medium is available as a premixed powder from Oxoid Unipath.

### Magnesium Chloride Solution:

**Composition per 100.0mL:**

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 40.0g

### Preparation of Magnesium Chloride Solution:

Add MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

### Malachite Green Solution:

**Composition per 10.0mL:**

Malachite Green oxalate ..... 0.04g

### Preparation of Malachite Green Solution:

Add Malachite Green to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 10 psi pressure–115°C.

**Use:** For the isolation and cultivation of *Salmonella* species from environmental specimens.

## Rappaport-Vassiliadis R10 Broth

**Composition per liter:**

MgCl<sub>2</sub>, anhydrous ..... 13.4g  
NaCl ..... 7.2g  
Papaic digest of soybean meal ..... 4.54g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.45g  
Malachite Green oxalate ..... 0.036g

pH 5.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into screw-capped tubes in 10.0mL volumes. Autoclave for 15 min at 10 psi pressure–116°C.

**Use:** For the isolation and cultivation of *Salmonella* species from environmental specimens.

## Rappaport-Vassiliadis Soya Peptone Broth (RVS Broth)

**Composition per liter:**

MgCl<sub>2</sub>, anhydrous ..... 13.58g  
NaCl ..... 7.2g  
Papaic digest of soybean meal ..... 4.5g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.26g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.18g  
Malachite Green ..... 0.036g

pH 5.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into screw-capped tubes in 10.0mL volumes. Autoclave for 15 min at 10 psi pressure–115°C.

**Use:** For the isolation and cultivation of *Salmonella* species from environmental specimens.

## Raymond's Medium

**Composition per liter:**

Na<sub>2</sub>HPO<sub>4</sub> ..... 3.0g  
NaCl ..... 3.0g  
NH<sub>4</sub>NO<sub>3</sub> ..... 2.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 2.0g

MgSO<sub>4</sub> ..... 0.2g  
Na<sub>2</sub>CO<sub>3</sub> ..... 0.1g  
MnSO<sub>4</sub> ..... 0.02g  
CaCl<sub>2</sub> ..... 0.01g  
FeSO<sub>4</sub> ..... 0.01g  
*n*-Hexadecane ..... 10.0mL

pH 6.8–7.0 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodococcus erythropolis*, *Rhodococcus luteus*, *Rhodococcus maris*, and other bacteria that can utilize *n*-hexadecane as a carbon source.

## RF Medium

**Composition per liter:**

Yeast extract ..... 0.05g  
Peptone ..... 0.05g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.05g  
L-Cysteine·HCl·H<sub>2</sub>O ..... 0.05g  
Salt solution ..... 50.0mL  
Rumen fluid, clarified ..... 30.0mL  
Resazurin (1% solution) ..... 0.1mL

pH 7.4 ± 0.2 at 25°C

## Salt Solution:

**Composition per liter:**

NaHCO<sub>3</sub> ..... 10.0g  
NaCl ..... 2.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
CaCl<sub>2</sub>, anhydrous ..... 0.2g  
MgSO<sub>4</sub> ..... 0.2g

**Preparation of Salts Solution:** Add CaCl<sub>2</sub> and MgSO<sub>4</sub> to 300.0mL of distilled/deionized water. Mix thoroughly until dissolved. Bring volume to 800.0mL with distilled/deionized water. Add remaining components while stirring. Bring volume to 1.0L. Mix thoroughly. Store at 4°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.2–6.3 with 4N HCl. Gently heat and bring to boiling under 100% N<sub>2</sub>. Anaerobically distribute into tubes in 7.0mL volumes. Cap with rubber stoppers. Place tubes in a press. Autoclave for 20 min at 15 psi pressure–121°C with fast exhaust. The pH of the medium should be 7.4 after autoclaving.

**Use:** For the cultivation and maintenance of *Treponema bryantii*.

## RGCA Medium

**Composition per 300.3mL:**

Rumen fluid ..... 120.0mL  
Solution IV ..... 65.0mL  
Mineral solution I ..... 45.0mL  
Mineral solution II ..... 45.0mL  
Na<sub>2</sub>CO<sub>3</sub> solution ..... 20.0mL  
L-Cysteine·HCl·H<sub>2</sub>O solution ..... 5.0mL  
Solution III ..... 0.3mL

pH 6.6 ± 0.2 at 25°C

## Mineral Solution I:

**Composition per 100.0mL:**

K<sub>2</sub>HPO<sub>4</sub> ..... 0.3g

**Preparation of Mineral Solution I:** Add  $K_2HPO_4$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Mineral Solution II:

**Composition** per 100.0mL:

$(NH_4)_2SO_4$ .....	0.6g
NaCl.....	0.6g
$K_2HPO_4$ .....	0.3g
$MgSO_4$ .....	0.06g
$CaCl_2$ .....	0.06g

**Preparation of Mineral Solution II:** Add  $K_2HPO_4$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Solution III:

**Composition** per 10.0mL:

Resazurin.....	0.01g
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**Preparation of Solution III:** Add resazurin to 10.0mL of distilled/deionized water. Mix thoroughly.

#### Solution IV:

**Composition** per 65.0mL:

Agar.....	4.5g
Glucose.....	0.6g
Cellobiose.....	0.6g

**Preparation of Solution IV:** Add components to distilled/deionized water and bring volume to 65.0mL. Mix thoroughly.

#### L-Cysteine-HCl-H<sub>2</sub>O Solution:

**Composition** per 100.0mL:

L-Cysteine-HCl-H <sub>2</sub> O.....	3.0g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Na<sub>2</sub>CO<sub>3</sub> Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	6.0g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Rumen Fluid:

**Composition** per 120.0mL:

Rumen fluid.....	120.0mL
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**Preparation of Rumen Fluid:** Filter rumen contents, obtained from a cow on an alfalfa-hay concentrate ration, through two layers of cheesecloth to remove larger particles. Store under CO<sub>2</sub> in quart milk bottles in the refrigerator. Much of the particulate matter settles out. Use the supernatant fluid.

**Preparation of Medium:** Combine 45.0mL of mineral solution I, 45.0mL of mineral solution II, 0.3mL of solution III, and 65.0mL of solution IV in a 500.0mL flask. Gently heat and bring to boiling. Add 120.0mL of rumen fluid. Gently heat and bring to boiling under 100% CO<sub>2</sub>. Cap with a rubber stopper and wire the stopper secure. Autoclave for 20 min at 15 psi pressure-121°C. Cool to 45°-50°C. Remove stopper and gas with 100% CO<sub>2</sub> to eliminate O<sub>2</sub>. Aseptically add 5.0mL of sterile L-cysteine-HCl-H<sub>2</sub>O solution and 20.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into tubes under 100% CO<sub>2</sub> in 6.0mL volumes. Cap with rubber stoppers.

**Use:** For the cultivation and maintenance of *Ruminococcus albus*, *Ruminococcus flavifaciens*, and *Succinimonas amylytica*.

#### Rhamnose Salts Medium

**Composition** per liter:

Rhamnose.....	10.0g
Yeast extract.....	3.0g
$K_2HPO_4$ .....	2.9g
$KH_2PO_4$ .....	2.1g
$NH_4Cl$ .....	2.0g
$MgSO_4 \cdot 7H_2O$ .....	0.4g
NaCl.....	30.0mg
$CaCl_2$ .....	3.0mg
$FeSO_4 \cdot 7H_2O$ .....	1.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C.

**Use:** For the cultivation of *Rhodococcus chlorophenolicus*.

#### Rhizobium Agar (LMG 201)

**Composition** per liter:

Agar.....	20.0g
Mannitol.....	10.0g
Yeast extract.....	1.0g
Sodium glutamate.....	0.5g
$KH_2PO_4$ .....	0.5g
$MgSO_4 \cdot 7H_2O$ .....	0.1g
$CaCl_2 \cdot 2H_2O$ .....	40.0mg
$FeCl_3$ .....	4.0mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure-121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Rhizobium fredii*, *Rhizobium galegae*, *Rhizobium leguminosarum*, *Rhizobium loti*, *Rhizobium meliloti*, and *Rhizobium tropici*.

#### Rhizobium BIII Defined Agar

**Composition** per liter:

Agar.....	13.0g
Mannitol.....	10.0g
Sodium glutamate.....	1.1g
$K_2HPO_4$ .....	0.23g
$MgSO_4 \cdot 7H_2O$ .....	0.1g
Trace elements stock.....	1.0mL
Vitamin stock.....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Stock:

**Composition** per liter:

Nitilotriacetic acid.....	7.0g
$CaCl_2 \cdot 2H_2O$ .....	6.62g
$H_3BO_3$ .....	0.145g
$FeSO_4 \cdot 7H_2O$ .....	0.125g
$Na_2MoO_4$ .....	0.125g
$ZnSO_4 \cdot 7H_2O$ .....	0.108g
$CoSO_4 \cdot 7H_2O$ .....	0.07g
$CuSO_4 \cdot 5H_2O$ .....	5.0mg
$MnCl_2 \cdot 4H_2O$ .....	4.3mg

**Preparation of Trace Elements Stock:** Add each of the components to 500.0mL of distilled/deionized water in the following order:  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{H}_3\text{BO}_3$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and  $\text{Na}_2\text{MoO}_4$ . Adjust pH to 5.0. Add nitrilotriacetic acid. Bring volume to 1.0L with distilled/deionized water.

#### Vitamin Stock:

##### Composition per liter:

Inositol .....	0.12g
<i>p</i> -Aminobenzoic acid .....	0.02g
Biotin .....	0.02g
Calcium pantothenate .....	0.02g
Nicotinic acid .....	0.02g
Pyridoxine-HCl .....	0.02g
Riboflavin .....	0.02g
Thiamine-HCl .....	0.02g
Sodium phosphate buffer (50.0mM solution, pH 7.0) .....	1.0L

**Preparation of Vitamin Stock:** Combine components. Mix thoroughly. Filter sterilize. Store at 4°C in the dark.

**Preparation of Medium:** Add components, except vitamin stock, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 1.0mL of sterile vitamin stock. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Rhizobium* species from root nodules.

#### *Rhizobium japonicum* Agar

##### Composition per liter:

Agar .....	15.0g
Mannitol .....	10.0g
Yeast extract .....	1.0g
Soil extract .....	200.0mL

#### Soil Extract:

##### Composition per liter:

African Violet soil .....	77.0g
$\text{Na}_2\text{CO}_3$ .....	0.2g

**Preparation of Soil Extract:** Add components to 1.0L of tap water. Autoclave for 15 min at 15 psi pressure–121°C. Filter through Whatman filter paper. Bring volume to 1.0L with tap water.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bradyrhizobium japonicum*.

#### *Rhizobium* Medium 1

##### Composition per liter:

Agar .....	15.0g
Yeast extract .....	10.0g
$\text{K}_2\text{HPO}_4$ .....	0.5g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.2g
NaCl .....	0.2g
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .....	0.002g
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Add agar. Gently heat

and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of members of the Rhizobiaceae.

#### *Rhizobium* Medium 2

##### Composition per liter:

Agar .....	15.0g
Glycerol .....	4.6g
$\text{CaSO}_4$ .....	1.3g
$\text{K}_2\text{HPO}_4$ .....	1.0g
L-Arabinose .....	1.0g
Yeast extract .....	1.0g
$\text{KNO}_3$ .....	0.7g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.36g
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .....	4.0mg
pH 7.2 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of members of the Rhizobiaceae.

#### *Rhizobium* X Medium

##### Composition per liter:

Agar .....	15.0g
Mannitol .....	10.0g
Yeast extract .....	1.0g
Soil extract .....	200.0mL
pH 7.2 ± 0.2 at 25°C	

#### Soil Extract:

##### Composition per 200.0mL:

African Violet soil .....	77.0g
$\text{Na}_2\text{CO}_3$ .....	0.2g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 200.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman #1 filter paper.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bradyrhizobium japonicum*, *Rhizobium* species, and *Sinorhizobium xinjiangensis*.

#### *Rhizobium* X Medium with Thiram

##### Composition per liter:

Agar .....	15.0g
Mannitol .....	10.0g
Yeast extract .....	1.0g
Soil extract .....	200.0mL
Thiram solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

#### Thiram Solution:

##### Composition per 10.0mL:

Thiram .....	1.0mg
Ethanol, absolute .....	10.0mL

**Preparation of Thiram Solution:** Add thiram to 10.0mL of absolute ethanol. Mix thoroughly. Filter sterilize.

#### Soil Extract:

**Composition per 200.0mL:**

African Violet soil.....	77.0g
Na <sub>2</sub> CO <sub>3</sub> .....	0.2g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 200.0mL.

**Preparation of Medium:** Add components, except thiram solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL of sterile thiram solution. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Bradyrhizobium japonicum*, *Rhizobium* species, and *Sinorhizobium xinjiangensis*.

**Rhizoctonia Isolation Medium****Composition per liter:**

Agar .....	20.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KCl.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaNO <sub>2</sub> .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01g
Dexon® solution.....	10.0mL
Antibiotic solution .....	10.0mL
Gallic acid solution .....	10.0mL

**Antibiotic Solution:****Composition per 10.0mL:**

Chloramphenicol.....	0.05g
Streptomycin.....	0.05g

**Preparation of Antibiotic Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Dexon® Solution:****Composition per 10.0mL:**

Dexon (Chemagro®) wettable powder.....	0.09g
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**Preparation of Dexon Solution:** Add Dexon® to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Gallic Acid Solution:****Composition per 10.0mL:**

Gallic acid .....	0.4g
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**Preparation of Gallic Acid Solution:** Add gallic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Dexon solution, antibiotic solution, and gallic acid solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile Dexon solution, sterile antibiotic solution, and sterile gallic acid solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Rhizoctonia* species.

**Rhizomonas Medium****Composition per liter:**

Noble agar.....	11.0g
Pancreatic digest of casein .....	5.0g
Glucose .....	2.5g

K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
KNO <sub>3</sub> .....	0.5g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.06g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Rhizomonas suberifaciens*.

**Rhizomonas suberifaciens Medium****Composition per liter:**

Pancreatic digest of casein .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	1.3g
Agar, noble .....	1.1g
KNO <sub>3</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	60.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Rhizomonas suberifaciens*.

**Rhodobacter adriaticus Medium****Composition per 1001.0mL:**

NaCl .....	25.0g
NaHCO <sub>3</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Sodium ascorbate .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Trace elements solution SLA .....	1.0mL
Vitamin solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution SLA:****Composition per liter:**

CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	10.0g
FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	10.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	10.0mg

**Preparation of Trace Elements Solution SLA:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Bring pH to 2.0–3.0.

**Vitamin Solution:****Composition per liter:**

Nicotinamide .....	35.0mg
Thiamine·HCl .....	30.0mg
p-Aminobenzoic acid .....	20.0mg
Pyridoxal·HCl.....	10.0mg
Calcium DL-pantothenate .....	10.0mg
Biotin .....	10.0mg
Vitamin B <sub>12</sub> .....	5.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Rhodobacter adraiticus*.

### ***Rhodobacter Medium* (LMG Medium 80)**

**Composition** per liter:

Yeast extract .....	1.0g
Di-sodium succinate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
Ferric citrate solution .....	5.0mL
Trace elements solution .....	1.0mL
Ethanol .....	0.5mL

pH 5.8 ± 0.2 at 25°C

**Ferric Citrate Solution :**

**Composition** per 100.0mL:

Ferric citrate .....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Trace Elements Solution:**

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute 40mL medium into 50mL screw-capped bottles. Flush each bottle for 1 to 2 min with nitrogen gas and then close immediately with a rubber septum and screw-cap. Autoclave for 15 min at 15 psi pressure–121°C. Sterile syringes are used to inoculate and remove the samples. Incubate in light using a tungsten lamp.

**Use:** For the cultivation of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, and *Rhodospirillum rubrum*.

### ***Rhodobacter veldkampii* Medium (DSMZ Medium 867)**

**Composition** per 2780mL:

Solution 1 .....	1540.0mL
Solution 3 .....	1000.0mL
Solution 4 .....	120.0mL
Solution 5 .....	120.0mL

pH 4.0 ± 0.1 at 25°C

**Solution 1:**

**Composition** per 2500mL:

CaCl <sub>2</sub> .....	2.0g
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**Preparation of Solution 1:** Add CaCl<sub>2</sub> to distilled/deionized water and bring volume to 2.5L. Mix thoroughly.

**Solution 3:**

**Composition** per liter:

NaHCO <sub>3</sub> .....	4.5g
Solution 2 .....	100.0mL

**Preparation of Solution 3:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with gaseous CO<sub>2</sub> through for at least 30 min. Add 100.0mL solution 2. Immediately filter sterilize using CO<sub>2</sub> pressure to push the liquid through (no suction).

**Solution 2:**

**Composition** per 100mL:

Sodium ascorbate .....	2.4g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
KCl .....	1.0g
NH <sub>4</sub> Cl .....	0.8g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.8g
Heavy metal solution .....	50.0mL
Vitamin solution .....	15.0mL
Vitamin B <sub>12</sub> solution .....	3.0mL

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Heavy Metal Solution:**

**Composition** per liter:

EDTA .....	1.50g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.02g
Modified Hoagland trace elements solution .....	6.0mL

**Preparation of Heavy Metal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Modified Hoagland Trace Elements Solution:**

**Composition** per 3.6L:

H <sub>3</sub> BO <sub>3</sub> .....	11.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	7.0g
ZnCl <sub>2</sub> .....	1.0g
CuCl <sub>2</sub> .....	1.0g
NiCl <sub>2</sub> .....	1.0g
CoCl <sub>2</sub> .....	1.0g
AlCl <sub>3</sub> .....	1.0g
KI .....	1.0g
KBr .....	0.5g
LiCl .....	0.5g
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
BaCl <sub>2</sub> .....	0.5g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.5g
NaVO <sub>3</sub> ·H <sub>2</sub> O .....	0.1g

**Preparation of Modified Hoagland Trace Elements Solution:** Add components sequentially to distilled/deionized water and bring final volume to 3.6L. Mix thoroughly after adding each component until dissolved. Adjust pH to just below 7.0. Adjust the final pH to 3–4. The flaky yellow precipitate which is formed after mixing transforms after standing for one or a few days into a very fine white precipitate. Mix thoroughly before use.

**Vitamin B<sub>12</sub> Solution:****Composition** per 3mL:

Vitamin B<sub>12</sub> (cyanocobalamine) ..... 2.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 3.0mL. Mix thoroughly.

**Vitamin Solution:****Composition** per 100mL:

Pyridoxamine·2HCl ..... 5.0mg  
 Nicotinic acid ..... 2.0mg  
 Thiamine ..... 1.0mg  
 Pantothenic acid ..... 0.5mg  
 Biotin ..... 0.2mg  
*p*-Aminobenzoic acid ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub> for 30 min.

**Solution 4:****Composition** per 200mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 3.0g

**Preparation of Solution 4:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a flask with a magnetic stirrer and bring volume to 200.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature. Partially neutralize the sterilized solution by adding, on a magnetic stirrer, drop by drop, 2.0mL sterile 2*M* H<sub>2</sub>SO<sub>4</sub>.

**Solution 5:****Composition** per 200mL:

Na-acetate ..... 6.0g

**Preparation of Solution 5:** Add Na-acetate to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 5 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Distribute solution 1 in 77.0mL amounts into 20 127mL screw-capped Boston round bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 50.0mL sterile solution 3 to each of the 20 127mL bottles containing 77.0mL of sterile solution 1 so that the solution completely fills the bottle. Mix thoroughly. Remove 6.0mL of the medium from the completely filled bottles. Add 6.0mL of neutralized solution 4 so that the bottles are again completely filled. Mix thoroughly. Remove 6.0mL of the medium from the completely filled bottles. Add 6.0mL of solution 5 so that the bottles are again completely filled. Mix thoroughly. Adjust pH to 4.0. Allow the bottles to stand overnight to develop a hazy, white precipitate before inoculating. Mix the solution thoroughly before use. To inoculate, remove 6.0mL of completed medium and replace it with an equal volume of inoculum. Grow cultures under tungsten light.

**Use:** For the cultivation of *Rhodobacter veldkampii*.

**Rhodobium Medium  
(DSMZ Medium 745)**

**Composition** per liter:

NaCl ..... 50.2g  
 Na-DL-malate ..... 3.6g  
 Yeast extract ..... 1.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g

Na<sub>2</sub>S·9H<sub>2</sub>O solution ..... 10.0mL  
 Trace elements solution SL-8 ..... 1.0mL  
 pH 6.8 ± 0.2 at 25°C

**Trace Elements Solution SL-8:****Composition** per liter:

Na<sub>2</sub>-EDTA ..... 5.2g  
 FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl<sub>2</sub> ..... 70.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 62.0mg  
 Na<sub>2</sub>MoSO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 17.0mg

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Adjust pH to 6.8. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust to pH 6.8. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Add 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically under 100% N<sub>2</sub> distribute into sterile screw-cap tubes or flasks.

**Use:** For the cultivation of *Rhodobium orientis* (*Rhodovulum orientis*).

**Rhodocyclus Medium  
(LMG Medium 82)**

**Composition** per liter:

Yeast extract ..... 1.0g  
 Ammonium acetate ..... 0.5g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.5g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.4g  
 NaCl ..... 0.4g  
 NH<sub>4</sub>Cl ..... 0.4g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 50.0mg  
 Ferric citrate solution ..... 5.0mL  
 Trace elements solution ..... 1.0mL  
 Vitamin solution ..... 1.0mL  
 Ethanol ..... 0.5mL  
 pH 5.8 ± 0.2 at 25°C

**Ferric Citrate Solution:****Composition** per 100.0mL:

Ferric citrate ..... 0.1g

**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Trace Elements Solution:****Composition** per liter:

H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Vitamin Solution:**

**Composition** per 100.0mL:

Vitamin B <sub>12</sub> .....	2.0mg
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**Preparation of Vitamin Solution:** Add Vitamin B<sub>12</sub> to 100.0mL of distilled/deionized water. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute 40.0mL medium into 50mL screw-capped bottles. Flush each bottle for 1 to 2 min with nitrogen gas and then close immediately with rubber septa and screw-caps. Autoclave for 15 min at 15 psi pressure–121°C. Sterile syringes are used to inoculate and remove the samples. Incubate in light using a tungsten lamp.

**Use:** For the cultivation of *Rhodocyclus purpureus*.

### ***Rhodocyclus purpureus* Medium (DSMZ Medium 44)**

**Composition** per 1056.9 mL:

Na <sub>2</sub> -succinate .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> -acetate .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
Yeast extract .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Ferric citrate solution .....	5.0mL
Trace elements solution SL-6 .....	1.0mL
Ethanol .....	0.5mL
Vitamin B <sub>12</sub> solution .....	0.4mL
Sulfide solution .....	variable

pH 6.8 ± 0.2 at 25°C

#### **Trace Elements Solution SL-6:**

**Composition** per liter:

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Vitamin B<sub>12</sub> Solution:**

**Composition** per 100.0mL:

Vitamin B <sub>12</sub> .....	10.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min.

#### **Ferric Citrate Solution:**

**Composition** per 10.0mL:

Ferric citrate .....	10.0mg
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to

10.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min.

#### **Neutralized Sulfide Solution:**

**Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.5g
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#### **Preparation of Neutralized Sulfide Solution:**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.0 with sterile 2M H<sub>2</sub>SO<sub>4</sub>. Do not open the bottle to add H<sub>2</sub>SO<sub>4</sub>; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add components, except neutralized sulfide solution, to 1050.0mL distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Boil for 3–4 min under a stream of 100% N<sub>2</sub>. Distribute 45.0mL of the prepared medium into 50.0mL screw-capped tubes that have been flushed with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Before inoculation, aseptically and anaerobically add 0.25–0.50mL of neutralized sulfide solution. Sterile syringes are used to inoculate and remove samples. Incubate in the light using a tungsten lamp.

**Use:** For the cultivation and maintenance of brown and other oxygen sensitive Rhodospirillaceae.

### ***Rhodomicrobium* Medium (LMG Medium 79)**

**Composition** per liter:

Di-sodium succinate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
Yeast extract .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
Ferric citrate solution .....	5.0mL
Trace elements solution .....	1.0mL

pH 5.7 ± 0.2 at 25°C

#### **Ferric Citrate Solution :**

**Composition** per 100.0mL:

Ferric citrate .....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **Trace Elements Solution:**

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute 40.0mL medium into 50mL screw-capped bot-

ties. Flush each bottle for 1 to 2 min with nitrogen gas and then close immediately with rubber septa and screw-caps. Autoclave for 15 min at 15 psi pressure–121°C. Sterile syringes are used to inoculate and remove the samples. Incubate in light using a tungsten lamp.

**Use:** For the cultivation of *Rhodomicrobium vannielii* and *Rhodoblastus acidophilus*.

### ***Rhodopila globiformis* Medium**

#### **Composition per liter:**

Mannitol	1.5g
Sodium gluconate	0.56g
KH <sub>2</sub> PO <sub>4</sub>	0.4g
NaCl	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.4g
NH <sub>4</sub> Cl	0.4g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Ferric citrate	5.0mg
VA vitamins	1.0mL
Trace elements solution SL-6	1.0mL
pH 4.9 ± 0.2 at 25°C	

#### **VA Vitamins:**

##### **Composition per 500.0mL:**

Nicotinamide	0.175g
Thiamine-HCl	0.15g
<i>p</i> -Aminobenzoic acid	0.1g
Biotin	0.05g
Pyridoxine-2HCl	0.05g
Calcium pantothenate	0.05g
Cyanocobalamin	0.025g

**Preparation of VA Vitamins:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

#### **Trace Elements Solution SL-6:**

##### **Composition per liter:**

H <sub>3</sub> BO <sub>3</sub>	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4.9. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodopila globiformis*.

### ***Rhodopseudomonas blastica* Medium**

#### **Composition per liter:**

Sodium pyruvate	1.5g
Sodium hydrogen malate	1.5g
Yeast extract	1.0g
NH <sub>4</sub> Cl	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4g
NaCl	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Sodium phosphate buffer (0.1M, pH 6.8)	50.0mL
pH 6.8 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except sodium pyruvate solution, sodium hydrogen malate solution, and sodium phosphate buffer, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.8 with KOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Filter sterilize the sodium pyruvate solution, sodium hydrogen malate solution, and sodium phosphate buffer solution. Aseptically add 1.5g of sterile sodium pyruvate solution, 1.5g of sodium hydrogen malate solution, and 50.0mL of sodium phosphate buffer solution to cooled basal medium. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Rhodopseudomonas blastica* and other *Rhodopseudomonas* species.

### ***Rhodopseudomonas globiformis* Medium**

#### **Composition per 1002.0mL:**

Mannitol	1.5g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
Sodium gluconate	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4g
NaCl	0.4g
NH <sub>4</sub> Cl	0.4g
Yeast extract	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Ferric citrate solution	5.0mL
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution	2.0mL
Biotin solution	1.0mL
<i>p</i> -Aminobenzoic acid solution	1.0mL
Trace elements solution SL-6	1.0mL
pH 4.9 ± 0.2 at 25°C	

#### **Ferric Citrate Solution :**

##### **Composition per 10.0mL:**

Ferric citrate	0.01g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution :**

##### **Composition per 10.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	1.0g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Biotin Solution:**

##### **Composition per 10.0mL:**

Biotin	0.2mg
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**Preparation of Biotin Solution:** Add biotin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### ***p*-Aminobenzoic Acid Solution:**

##### **Composition per 10.0mL:**

<i>p</i> -Aminobenzoic acid	1.0mg
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**Preparation of *p*-Aminobenzoic Acid Solution:** Add *p*-aminobenzoic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Elements Solution SL-6:**

##### **Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.5g
H <sub>3</sub> BO <sub>3</sub>	0.3g



CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, ferric citrate solution, biotin solution, and *p*-aminobenzoic acid solution, to distilled deionized water and bring volume to 993.0mL. Mix thoroughly. Adjust pH to 4.9. Add 5.0mL of ferric citrate solution, 1.0mL of biotin solution, and 1.0mL of *p*-aminobenzoic acid solution. Mix thoroughly. Distribute into screw-capped tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Allow to cool to room temperature. Aseptically add 0.2mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution to each 100.0mL of medium. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Rhodopila globiformis*.

#### *Rhodopseudomonas julia* Medium

##### Composition per liter:

NaHCO <sub>3</sub> .....	3.0g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	1.0g
Sodium acetate .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	0.7g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Sodium ascorbate .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Yeast extract .....	0.1g
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
SLA trace elements solution .....	1.0mL
VA vitamin solution .....	1.0mL

pH 6.9–7.0 at 25°C

##### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.156g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

##### SLA Trace Elements Solution:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.25g
ZnCl <sub>2</sub> .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	10.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.0mg

**Preparation of SLA Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2.0–3.0.

##### VA Vitamin Solution:

##### Composition per 500.0mL:

Nicotinamide .....	0.175g
Thiamine·HCl .....	0.15g

<i>p</i> -Aminobenzoic acid .....	0.1g
Biotin .....	50.0mg
Calcium D-(+)-pantothenate .....	50.0mg
Pyridoxine·2HCl .....	50.0mg
Cyanocobalamin .....	25.0mg

**Preparation of VA Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 6.9–7.0. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Rhodopseudomonas julia*.

#### *Rhodopseudomonas* Medium (ATCC Medium 543)

##### Composition per liter:

Sodium succinate .....	2.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.25g
K <sub>2</sub> HPO <sub>4</sub> .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g
Yeast extract .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.07g
Ferric citrate .....	3.0mg
Ethylenediamine tetraacetate (EDTA) .....	2.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodopseudomonas* species.

#### *Rhodopseudomonas rutila* Medium

##### Composition per liter:

Sodium glutamate .....	2.0g
Sodium L-malate .....	2.0g
Yeast extract .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NaHCO <sub>3</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg
Biotin .....	1.0mg
Nicotinic acid .....	1.0mg
Thiamine·HCl .....	1.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodopseudomonas palustris* and *Rhodopseudomonas rutila*.

#### *Rhodopseudomonas sulfoviridis* Medium

##### Composition per 1050.0 mL:

Ammonium acetate .....	1.5g
Sodium malate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	0.5g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
Yeast extract .....	0.3g
Disodium succinate .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Ferric citrate solution .....	5.0mL
Trace elements solution SL-6 .....	1.0mL
Ethanol .....	0.5mL
Vitamin B <sub>12</sub> solution .....	0.4mL
Neutralized sulfide solution .....	variable
pH 6.8 ± 0.2 at 25°C	

**Ferric Citrate Solution:****Composition per 10.0mL:**

Ferric citrate .....	10.0mg
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub>.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	10.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub>.

**Neutralized Sulfide Solution:****Composition per 100.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	1.5g
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**Preparation of Neutralized Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100% N<sub>2</sub> for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.3 with sterile 2M H<sub>2</sub>SO<sub>4</sub>. Do not open the bottle to add H<sub>2</sub>SO<sub>4</sub>; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add components, except neutralized sulfide solution, to distilled/deionized water and bring volume to 1050.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3–4 min under a stream of 100% N<sub>2</sub>. Distribute 45.0mL of the prepared medium into 50.0mL screw-capped tubes that have been flushed with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Before inoculation, aseptically and anaerobically add 0.25–0.50mL of neutralized sulfide solution to each tube.

**Use:** For the cultivation and maintenance of *Rhodopseudomonas sulfoviridis*.

**Rhodospirillaceae Enrichment Medium****Composition per liter:**

Dicarboxylic acid substrate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Yeast extract .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Ferric citrate solution .....	5.0mL
Trace elements solution SL-7 .....	1.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL
pH 6.8 ± 0.2 at 25°C	

**Ferric Citrate Solution:****Composition per 100.0mL:**

Ferric citrate .....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Trace Elements Solution SL-7:****Composition per liter:**

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
HCl (25% solution) .....	1.0mL

**Preparation of Trace Elements Solution SL-7:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:****Composition per 100.0mL:**

Vitamin B <sub>12</sub> .....	1.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Succinic acid or glutaric acid may be used for the dicarboxylic acid substrate. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enrichment and isolation of members of the Rhodospirillaceae.

**Rhodospirillaceae Medium****Composition per liter:**

Succinic acid .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Yeast extract .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Ferric citrate solution .....	5.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL
Trace elements solution SL7 .....	1.0mL
pH 6.8 ± 0.2 at 25°C	

**Ferric Citrate Solution:****Composition** per 100.0mL:

Ferric citrate ..... 0.1g

**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Vitamin B<sub>12</sub> Solution:****Composition** per 100.0mL:

Vitamin B<sub>12</sub> ..... 1.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize

**Trace Elements Solution SL7:****Composition** per liter:

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
 ZnCl ..... 70.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 60.0mg  
 NaMoO<sub>4</sub>·2H<sub>2</sub>O ..... 40.0mg  
 CoCl<sub>2</sub>·2H<sub>2</sub>O ..... 20.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 20.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 20.0mg  
 HCl (25%) ..... 1.0mL

**Preparation of Trace Elements Solution SL7:** Add MnCl<sub>2</sub>·4H<sub>2</sub>O to 1.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Ferric citrate solution, Vitamin B<sub>12</sub> solution, and trace elements solution SL7, to distilled/deionized water and bring volume to 993.0mL. Mix thoroughly. Adjust pH to 6.8. Autoclave for 30 min at 15 psi pressure–121°C. Aseptically add 5.0mL of sterile ferric citrate solution, 1.0mL of sterile Vitamin B<sub>12</sub> solution, and 1.0mL of sterile trace elements solution SL7. Mix thoroughly. Aseptically distribute into sterile screw-capped tubes under anaerobic condition. Tighten screw caps.

**Use:** For the cultivation of *Rhodospirillum* species and other members of the family Rhodospirillaceae.

**Rhodospirillaceae Medium, Modified****Composition** per 1050.0 mL:

Ammonium acetate ..... 1.5g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.5g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.4g  
 NaCl ..... 0.4g  
 NH<sub>4</sub>Cl ..... 0.4g  
 Yeast extract ..... 0.3g  
 Disodium succinate ..... 0.25g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g  
 Ferric citrate solution ..... 5.0mL  
 Trace elements solution SL-6 ..... 1.0mL  
 Ethanol ..... 0.5mL  
 Vitamin B<sub>12</sub> solution ..... 0.4mL  
 Neutralized sulfide solution ..... variable

pH 6.8 ± 0.2 at 25°C

**Ferric Citrate Solution:****Composition** per 10.0mL:

Ferric citrate ..... 10.0mg

**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

**Trace Elements Solution SL-6:****Composition** per liter:

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.5g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.03g  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02g  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Vitamin B<sub>12</sub> Solution:****Composition** per 100.0mL:

Vitamin B<sub>12</sub> ..... 10.0mg

**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub> gas.

**Neutralized Sulfide Solution:****Composition** per 100.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 1.5g

**Preparation of Neutralized Sulfide Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water in a 250mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.3 with sterile 2M H<sub>2</sub>SO<sub>4</sub>. Do not open the bottle to add H<sub>2</sub>SO<sub>4</sub>; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow in color.

**Preparation of Medium:** Add components, except neutralized sulfide solution, to distilled/deionized water and bring volume to 1050.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3–4 min under a stream of 100% N<sub>2</sub>. Distribute 45.0mL of the prepared medium into 50.0mL screw-capped tubes that have been flushed with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Before inoculation, aseptically and anaerobically add 0.25–0.50mL of neutralized sulfide solution to each tube.

**Use:** For the cultivation and maintenance of *Ectothiorhodospira marismortui*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rhodobacter sulfidophilus*, *Rhodocyclus tenuis*, *Rhodopseudomonas blastica*, *Rhodopseudomonas marina*, *Rhodopseudomonas palustris*, *Rhodopseudomonas rosea*, *Rhodopseudomonas viridis*, *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, *Rhodospirillum photometricum*, *Rhodospirillum rubrum*, *Rhodospirillum salexigens*, and *Rubrivivax gelatinosus*.

**Rhodospirillum Medium  
(ATCC Medium 1308)****Composition** per liter:

Yeast extract ..... 1.0g  
 Disodium succinate ..... 1.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 0.5g  
 Sodium ascorbate ..... 0.5g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.4g  
 NaCl ..... 0.4g  
 NH<sub>4</sub>Cl ..... 0.4g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.05g  
 Ferric citrate (0.1% solution) ..... 5.0mL

Trace elements solution SL-6 .....	1.0mL
Ethanol .....	0.5mL
pH $6.0 \pm 0.2$ at 25°C	

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodospirillum* species.

**Rhodospirillum Medium, Modified I****Composition per liter:**

Disodium succinate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
Yeast extract .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Ferric citrate (0.1% solution) .....	5.0mL
Trace elements solution SL-6 .....	1.0mL
pH $5.7 \pm 0.2$ at 25°C	

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.10g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.7. Distribute 40.0mL volumes into tubes or bottles. Sparge with 100% N<sub>2</sub> for 1–2 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodomicrobium vannielii*, *Rhodopseudomonas acidophila*, and *Rhodobacter capsulatus*.

**Rhodospirillum Medium, Modified II****Composition per liter:**

Yeast extract .....	1.0g
Disodium succinate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Ferric citrate (0.1% solution) .....	5.0mL

Trace elements solution SL-6 .....	1.0mL
Ethanol .....	0.5mL
pH $6.8 \pm 0.2$ at 25°C	

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.10g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute 40.0mL volumes into tubes or bottles. Sparge with 100% N<sub>2</sub> for 1–2 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodobacter sphaeroides*, *Rhodocyclus tenuis*, *Rhodopseudomonas blastica*, *Rhodopseudomonas palustris*, *Rhodopseudomonas viridis*, *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, *Rhodospirillum photometricum*, *Rhodospirillum rubrum*, and *Rubrivivax gelatinosus*.

**Rhodospirillum Medium, Modified III****Composition per liter:**

Yeast extract .....	1.0g
Ammonium acetate .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Vitamin B <sub>12</sub> .....	20.0mg
Ferric citrate (0.1% solution) .....	5.0mL
Trace elements solution SL-6 .....	1.0mL
Ethanol .....	0.5mL
pH $6.8 \pm 0.2$ at 25°C	

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute 40.0mL volumes into tubes or bottles. Sparge with 100% N<sub>2</sub> for 1–2 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodocyclus purpureus*.

**Rhodospirillum Medium, Modified IV****Composition per liter:**

NaCl .....	25.0g
Yeast extract .....	1.0g

Disodium succinate.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4g
NaCl.....	0.4g
NH <sub>4</sub> Cl.....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
Ferric citrate (0.1% solution).....	5.0mL
Trace elements solution SL-6.....	1.0mL
Ethanol.....	0.5mL

pH 6.8 ± 0.2 at 25°C

**Trace Elements Solution SL-6:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute 40.0mL volumes into tubes or bottles. Sparge with 100% N<sub>2</sub> for 1–2 min. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Rhodobacter sulfidophilus*.

***Rhodospirillum salinarum* Medium****Composition per liter:**

NaCl.....	100.0g
KCl.....	5.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0g
NH <sub>4</sub> Cl.....	5.0g
Peptone solution.....	30.0mL
Yeast extract solution.....	30.0mL
Ferric citrate solution.....	10.0mL
Trace elements solution.....	5.0mL

**Peptone Solution:****Composition per 100.0mL:**

Peptone.....	15.0g
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**Preparation of Peptone Solution:** Add peptone to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Yeast Extract Solution:****Composition per 10.0mL:**

Yeast extract.....	15.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Ferric Citrate Solution:****Composition per 10.0mL:**

Ferric citrate.....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	10.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	220.0mg

MgCl <sub>2</sub> ·4H <sub>2</sub> O.....	180.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	6.3mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	1.0mg

**Preparation of Medium:** Add components, except peptone solution, yeast extract solution, ferric citrate solution, and trace elements solution to distilled/deionized water and bring volume to 925.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 30.0mL of sterile peptone solution, 30.0mL of sterile yeast extract solution, 10.0mL of sterile ferric citrate solution, and 5.0mL of sterile trace elements solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Rhodospirillum salinarum*.

***Rhodovulum iododum*  
*Rhodovulum robiginosum* Medium  
(DSMZ Medium 929)****Composition per liter:**

NaCl.....	26.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.8g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.7g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.5g
KCl.....	0.66g
KBr.....	0.09g
NaHCO <sub>3</sub> solution.....	30.0mL
Phosphate solution.....	10.0mL
Sodium acetate solution.....	10.0mL
Iron sulfate solution.....	10.0mL
NH <sub>4</sub> Cl solution.....	1.0mL
Thiosulfate solution.....	1.0mL
Selenite-tungstate solution.....	1.0mL
Trace elements solution.....	1.0mL
Vitamin solution.....	1.0mL
Vitamin B <sub>12</sub> solution.....	1.0mL
Vitamin B <sub>1</sub> solution.....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Sodium Acetate Solution:****Composition per 100.0mL:**

Na-acetate.....	4.1g
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**Preparation of Sodium Acetate Solution:** Add sodium acetate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	144.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	36.0mg
H <sub>3</sub> BO <sub>3</sub> .....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
Na <sub>2</sub> EDTA.....	5.2mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.1mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Vitamin Solution:****Composition per 100.0mL:**

Pyridoxin.....	15.0mg
Nicotinate.....	10.0mg
Pantothenate.....	5.0mg

Para-aminobenzoic acid .....	4.0mg
Biotin .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Vitamin B<sub>12</sub> Solution:

**Composition** per 100.0mL:

Cyanocobalamine.....	5.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add cyanocobalamine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Vitamin B<sub>1</sub> Solution:

**Composition** per 100.0mL:

Thiamine .....	10.0mg
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**Preparation of Vitamin B<sub>1</sub> Solution:** Add thiamine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### NH<sub>4</sub>Cl Solution:

**Composition** per 10.0mL:

NH <sub>4</sub> Cl.....	2.5g
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**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Thiosulfate Solution:

**Composition** per 10mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	1.24g
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**Preparation of Thiosulfate Solution:** Add 1.24g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### NaHCO<sub>3</sub> Solution:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	8.4g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C under an atmosphere of CO<sub>2</sub>. Cool to room temperature.

#### Selenite-Tungstate Solution

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Phosphate Solution:

**Composition** per 10mL:

KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
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**Preparation of Phosphate Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Iron Sulfate Solution:

**Composition** per 100mL:

FeSO <sub>4</sub> .....	1.52g
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**Preparation of Iron Sulfate Solution:** Add FeSO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under 90% N<sub>2</sub> + 10% CO<sub>2</sub> gas mixture. Add components, except NaHCO<sub>3</sub> solution, phosphate solution, sodium acetate solution, iron sulfate solution, NH<sub>4</sub>Cl solution, thiosulfate solution, selenite-tungstate solution, trace elements solution, vitamin solution, Vitamin B<sub>12</sub> solution, and Vitamin B<sub>1</sub> solution, to distilled/deionized water and bring volume to 933.0mL. Mix thoroughly. Sparge with 90% N<sub>2</sub> + 10% CO<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 30.0mL NaHCO<sub>3</sub> solution, 10.0mL phosphate solution, 10.0mL sodium acetate solution, 10.0mL iron sulfate solution, 1.0mL NH<sub>4</sub>Cl solution, 1.0mL thiosulfate solution, 1.0mL selenite-tungstate solution, 1.0mL trace elements solution, 1.0mL vitamin solution, 1.0mL Vitamin B<sub>12</sub> solution, and 1.0mL Vitamin B<sub>1</sub> solution. When the iron is added a white precipitate may form. Adjust pH to 6.8. Aseptically and anaerobically distribute into tubes or bottles.

**Use:** For the cultivation of *Rhodovulum iodolum* and *Rhodovulum robiginosum*.

#### *Rhodovulum strictum* Medium (DSMZ Medium 746)

**Composition** per liter:

Solution 1 .....	500.0mL
Solution 2 .....	500.0mL
pH 7.8 ± 0.2 at 25°C	

#### Solution 1:

**Composition** 500mL:

NaCl .....	8.2g
Na-DL-malate.....	3.6g
Yeast extract .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Trace elements solution SL-8.....	1.0mL

#### Trace Elements Solution SL-8:

**Composition** per liter:

Na <sub>2</sub> -EDTA .....	5.2g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
H <sub>3</sub> BO <sub>3</sub> .....	62.0mg
Na <sub>2</sub> MoSO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	17.0mg

**Preparation of Trace Elements Solution SL-8:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Solution 1:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 500.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 2:****Composition** per 500mL:

K <sub>2</sub> HPO <sub>4</sub> .....	1.35g
KH <sub>2</sub> PO <sub>4</sub> .....	0.35g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Aseptically and anaerobically combine 500.0mL solution 1 and 500.0mL solution 2 under N<sub>2</sub>. Mix thoroughly. Adjust pH to 7.8. Aseptically and anaerobically distribute into sterile screw cap tubes or flasks.

**Use:** For the cultivation of *Rhodovulum strictum*.

***Rhodovulum sulfidophilum* Medium  
(LMG Medium 84)**

**Composition** per liter:

NaCl .....	25.0g
Yeast extract .....	1.0g
Di-sodium succinate .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl.....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	50.0mg
Ferric citrate solution.....	5.0mL
Trace elements solution .....	1.0mL
Ethanol.....	0.5mL

pH 5.8 ± 0.2 at 25°C

**Ferric Citrate Solution:****Composition** per 100.0mL:

Ferric citrate.....	0.1g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Trace Elements Solution:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	30.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	20.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute 40.0mL medium into 50 mL screw-capped bottles. Flush each bottle for 1 to 2 minutes with nitrogen gas and then close immediately with a rubber septum and screw-cap. Autoclave for 15 min at 15 psi pressure–121°C. Sterile syringes are used to inoculate and remove the samples. Incubate in light using a tungsten lamp.

**Use:** For the cultivation of *Rhodovulum sulfidophilum*.

**Rila Marine Medium**

**Composition** per liter:

Agar .....	15.0g
Peptone.....	0.5g
Yeast extract.....	0.5g

Pancreatic digest of casein .....	0.5g
Marine salts mixture.....	800.0mL
pH 7.6–8.0 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 7.6–8.0. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Alteromonas denitrificans*.

**Rimler-Shotts Medium  
(RS Medium)**

**Composition** per liter:

Agar .....	13.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	6.8g
L-Ornithine·HCl.....	6.5g
NaCl .....	5.0g
L-Lysine·HCl .....	5.0g
Maltose .....	3.5g
Yeast extract .....	3.0g
Sodium deoxycholate .....	1.0g
Ferric ammonium citrate .....	0.8g
L-Cysteine·HCl.....	0.3g
Bromothymol Blue.....	0.03g
Novobiocin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Novobiocin Solution:****Composition** per 10.0mL:

Novobiocin .....	5.0mg
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**Preparation of Novobiocin Solution:** Add novobiocin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except novobiocin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile components. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation, cultivation, and presumptive identification of *Aeromonas hydrophila* and other Gram-negative bacteria based on their ability to decarboxylate lysine and ornithine, ferment maltose, and produce H<sub>2</sub>S. Maltose-fermenting bacteria appear as yellow colonies. Bacteria that produce lysine or ornithine decarboxylase turn the medium greenish-yellow to yellow. Bacteria that produce H<sub>2</sub>S appear as colonies with black centers.

**Rippey-Cabelli Agar  
(RC Agar)**

**Composition** per liter:

Agar .....	15.0g
Meat peptone .....	5.0g
Trehalose .....	5.0g
NaCl .....	3.0g
KCl .....	2.0g
Yeast extract .....	2.0g
Bromothymol Blue.....	0.44g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.1g
Sodium deoxycholate .....	0.1g
Ampicillin solution.....	10.0mL
Ethanol .....	10.0mL

pH 8.0 ± 0.2 at 25°C

**Ampicillin Solution:****Composition** per 10.0mL:

Ampicillin ..... 0.02g

**Preparation of Ampicillin Solution:** Add ampicillin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except sodium deoxycholate, ampicillin solution, and ethanol, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sodium deoxycholate, 10.0mL of sterile ampicillin solution, and 10.0mL of ethanol. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation, cultivation, and differentiation of *Aeromonas* species and *Plesiomonas* species from water samples using the membrane filter method. This medium differentiates bacteria on the basis of trehalose fermentation. Bacteria that ferment trehalose turn the medium yellow.

**RM Medium****Composition** per liter:

Glucose ..... 20.0g  
 Agar ..... 15.0g  
 Yeast extract ..... 10.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 2.0g  
 Solution 1 ..... 250.0mL  
 Solution 2 ..... 250.0mL  
 Solution 3 ..... 250.0mL  
 Solution 4 ..... 250.0mL

pH 6.0 ± 0.2 at 25°C

**Solution 1:****Composition** per 250.0mL:

Glucose ..... 20.0g

**Preparation of Solution 1:** Add glucose to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 2:****Composition** per 250.0mL:

Agar ..... 15.0g

**Preparation of Solution 2:** Add agar to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 3:****Composition** per 250.0mL:

Yeast extract ..... 10.0g

**Preparation of Solution 3:** Add yeast extract to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution 4:****Composition** per 250.0mL:KH<sub>2</sub>PO<sub>4</sub> ..... 2.0g

**Preparation of Solution 4:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine the four sterile solutions. Mix thoroughly. Adjust pH to 6.0. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Zymomonas mobilis*.

**Rose Bengal Chloramphenicol Agar****Composition** per liter:

Agar ..... 15.0g  
 Glucose ..... 10.0g  
 Papaic digest of soybean meal ..... 5.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 Rose Bengal ..... 0.05g  
 Chloramphenicol solution ..... 10.0mL

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Chloramphenicol Solution:****Composition** per 10.0mL:

Chloramphenicol ..... 0.1g

**Preparation of Chloramphenicol Solution:** Add chloramphenicol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except chloramphenicol solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°C. Aseptically add sterile chloramphenicol solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation, cultivation, and enumeration of yeasts and molds from environmental specimens.

**Roseinatronobacter Agar  
(DSMZ Medium 928)****Composition** per liter:

K<sub>2</sub>HPO<sub>4</sub> ..... 25.0g  
 Na<sub>2</sub>CO<sub>3</sub> ..... 11.0g  
 NaHCO<sub>3</sub> ..... 4.0g  
 NaCl ..... 2.5g  
 Sodium acetate ..... 0.8g  
 Yeast extract ..... 0.5g  
 Peptone ..... 0.5g  
 KNO<sub>3</sub> ..... 0.25g  
 Agar solution ..... 500.0mL

pH 10.0 ± 0.2 at 25°C

**Agar Solution:****Composition** per 500.0mL:

Agar ..... 20.0g

**Preparation of Agar Solution:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C.

**Preparation of Medium:** Add components, except agar solution, to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C. Add 500.0mL sterile warm agar solution. Mix thoroughly. Pour into Petri dishes or distribute to sterile tubes.

**Use:** For the cultivation of *Roseinatronobacter thiooxidans*.



**Rumen Bacteria Medium****Composition** per 1001.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	4.0g
Trypticase .....	2.0g
Yeast extract .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
Hemin .....	1.0mg
Resazurin .....	1.0mg
Minerals solution .....	38.0mL
Carbohydrate solution .....	20.0mL
L-Cysteine-HCl-H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL
Volatile fatty acid mixture .....	3.1mL

pH 6.7 ± 0.2 at 25°C

**Minerals Solution:****Composition** per liter:

NaCl .....	12.0g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.6g

**Preparation of Minerals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**L-Cysteine-HCl-H<sub>2</sub>O Solution:****Composition** per 10.0mL:

L-Cysteine-HCl-H <sub>2</sub> O .....	0.25g
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**Preparation of L-Cysteine-HCl-H<sub>2</sub>O Solution:** Add L-cysteine-HCl-H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S-9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S-9H <sub>2</sub> O .....	0.25g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Carbohydrate Solution:****Composition** per 20.0mL:

Glucose .....	0.5g
Cellobiose .....	0.5g
Glycerol .....	0.5g
Maltose .....	0.5g
Starch, soluble .....	0.5g

**Preparation of Carbohydrate Solution:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge under 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Volatile Fatty Acid Mixture:****Composition** per 7.75mL:

Acetic acid .....	4.25mL
Propionic acid .....	1.50mL
Butyric acid .....	1.0mL
DL-2-Methyl butyric acid .....	0.25mL
iso-Butyric acid .....	0.25mL
iso-Valeric acid .....	0.25mL
n-Valeric acid .....	0.25mL

**Preparation of Volatile Fatty Acid Mixture:** Combine components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% CO<sub>2</sub>. Add components, except carbohydrate solution, Na<sub>2</sub>CO<sub>3</sub>, L-cysteine-HCl-H<sub>2</sub>O solution, and Na<sub>2</sub>S-9H<sub>2</sub>O

solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 100% CO<sub>2</sub>. Add Na<sub>2</sub>CO<sub>3</sub>. Continue sparging with 100% CO<sub>2</sub> until pH reaches 6.8. Distribute into rubber-stoppered tubes under 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile carbohydrate solution, 10.0mL of sterile L-cysteine-HCl-H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution or, using a syringe, inject the appropriate amount of sterile carbohydrate solution, sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution, and sterile L-cysteine-HCl-H<sub>2</sub>O solution into individual tubes containing medium.

**Use:** For the cultivation and maintenance of *Anaerovibrio glycerini*, *Anaerovibrio lipolytica*, *Butyrivibrio fibrisolvens*, *Lachnospira multiparus*, *Succinimonas amylolytica*, and *Succinivibrio dextrinosolvens*.

**Rumen Fluid Cellobiose Agar**  
**(RFC Agar)**
**Composition** per 10.0mL:

Rumen fluid cellobiose base medium .....	8.9mL
NaHCO <sub>3</sub> -rifampin solution .....	1.0mL
Cellobiose solution .....	0.1mL

**Rumen Fluid Cellobiose Base Medium:****Composition** per 89.0mL:

Noble agar .....	0.7g
Cysteine-HCl-H <sub>2</sub> O .....	0.1g
Clarified rumen fluid .....	30.0mL
Salts solution A .....	20.0mL
Salts solution B .....	20.0mL
Resazurin (0.1% solution) .....	0.1mL

pH 6.7–7.0 at 25°C

**Preparation of Rumen Fluid Cellobiose Base Medium:** Add components to distilled/deionized water and bring volume to 89.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Anaerobically distribute into tubes in 8.9mL volumes under 100% CO<sub>2</sub>. Cap tubes with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Salts Solution A:****Composition** per liter:

CaCl <sub>2</sub> .....	0.45g
MgSO <sub>4</sub> .....	0.45g

**Preparation of Salts Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Salts Solution B:****Composition** per liter:

NaCl .....	4.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	4.5g
KH <sub>2</sub> PO <sub>4</sub> .....	2.25g
K <sub>2</sub> HPO <sub>4</sub> .....	2.25g

**Preparation of Salts Solution B:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**NaHCO<sub>3</sub>-Rifampin Solution:****Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	0.5g
Rifampin .....	0.1mg

**Preparation of NaHCO<sub>3</sub>-Rifampin Solution:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Cellobiose Solution:**

**Composition per 10.0mL:**

Cellobiose ..... 1.0g

**Preparation of Cellobiose Solution:** Add cellobiose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To each tube containing 8.9mL of sterile rumen fluid cellobiose base medium, aseptically add 1.0mL of sterile NaHCO<sub>3</sub>-rifampin solution and 0.1mL of sterile cellobiose solution. Mix thoroughly.

**Use:** For the selective isolation of rumen treponemes.

***Ruminobacter amylophilus* Medium**

**Composition per liter:**

Pancreatic digest of casein ..... 10.0g  
NaHCO<sub>3</sub> ..... 6.0g  
Starch, soluble ..... 5.0g  
NaCl ..... 0.9g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.9g  
L-Cysteine-HCl ..... 0.5g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.45g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.45g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.12g  
Resazurin ..... 1.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Prepare and dispense under 100% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Ruminobacter amylophilus*.

***Ruminococcus albus* Medium**

**Composition per 1001.0mL:**

Pancreatic digest of casein ..... 5.0g  
Na<sub>2</sub>CO<sub>3</sub> ..... 4.0g  
Glucose ..... 3.0g  
Cellobiose ..... 2.0g  
Yeast extract ..... 2.0g  
L-Cysteine-HCl ..... 0.5g  
Resazurin ..... 1.0mg  
Mineral solution 1 ..... 40.0mL  
Mineral solution 2 ..... 40.0mL  
Fatty acid mixture ..... 1.0mL

**Mineral Solution 1:**

**Composition per 100.0mL:**

K<sub>2</sub>HPO<sub>4</sub> ..... 0.6g

**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Mineral Solution 2:**

**Composition per 100.0mL:**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 2.0g  
NaCl ..... 2.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.6g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.25g  
CaCl<sub>2</sub>·7H<sub>2</sub>O ..... 0.16g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Fatty Acid Mixture:**

**Composition per 100.0mL:**

Isobutyric acid ..... 10.0mL  
Isovaleric acid ..... 10.0mL  
2-Methylbutyric acid ..... 10.0mL

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 100.0mL. Sparge with 100% CO<sub>2</sub>.

**Preparation of Medium:** Add components, except Na<sub>2</sub>CO<sub>3</sub>, L-cysteine-HCl, and fatty acid mixture, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 100% CO<sub>2</sub>. Add Na<sub>2</sub>CO<sub>3</sub>, L-cysteine-HCl, and fatty acid mixture. Adjust pH to 7.0. Anaerobically distribute into tubes or flasks under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Ruminococcus albus*.

***Ruminococcus pasteurii* Medium**

**Composition per liter:**

NaHCO<sub>3</sub> ..... 2.5g  
Sodium tartrate ..... 2.0g  
NaCl ..... 1.0g  
KCl ..... 0.5g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.4g  
Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.36g  
NH<sub>4</sub>Cl ..... 0.25g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g  
Resazurin ..... 1.0mg  
Trace elements solution SL-7 ..... 1.0mL

pH 7.2 ± 0.2 at 25°C

**Trace Elements Solution SL-7:**

**Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g  
ZnCl<sub>2</sub> ..... 0.07g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.062g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.036g  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.024g  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.017g  
HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-7:** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to the HCl. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C under 100% N<sub>2</sub>. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO<sub>3</sub> ..... 2.5g

**Preparation of NaHCO<sub>3</sub> Solution:** Add the NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.36g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C under 100% N<sub>2</sub>.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and trace elements solution SL-7, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into tubes in 9.8mL volumes under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Cool to 25°C. Aseptically add 0.1mL of sterile NaHCO<sub>3</sub> solution and 0.01mL of sterile trace elements solution SL-7 to each tube. Mix thoroughly. Immediately prior to inoculation, aseptically add 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube.

**Use:** For the cultivation and maintenance of *Ruminococcus pasteurii*.

### Russell Double-Sugar Agar

#### Composition per liter:

Agar .....	15.0g
Proteose peptone No. 3 .....	12.0g
Lactose .....	10.0g
NaCl .....	5.0g
Beef extract .....	1.0g
Glucose .....	1.0g
Phenol Red .....	0.025g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Use:** For the identification of Gram-negative enteric bacilli based on their fermentation of glucose and lactose. Bacteria that ferment both glucose and lactose produce a yellow slant and yellow butt. Bacteria that ferment glucose but do not ferment lactose produce a red slant and a yellow butt. Bacteria that ferment neither glucose nor lactose produce an unchanged pink-orange color.

### S6 Medium for Thiobacilli

#### Composition per liter:

Agar .....	15.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	11.8g
Na <sub>2</sub> HPO <sub>4</sub> .....	1.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
CaCl <sub>2</sub> .....	0.03g
FeCl <sub>3</sub> .....	0.02g
MnSO <sub>4</sub> .....	0.02g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus denitrificans* and *Thiobacillus thioparus*.

### S8 Medium for Thiobacilli

#### Composition per liter:

Agar .....	15.0g
KH <sub>2</sub> PO <sub>4</sub> .....	11.8g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	10.0g

KNO <sub>3</sub> .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	1.2g
NaHCO <sub>3</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
CaCl <sub>2</sub> .....	0.03g
FeCl <sub>3</sub> .....	0.02g
MnSO <sub>4</sub> .....	0.02g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus neapolitanus*.

### Sabouraud Agar

#### Composition per liter:

Neopeptone .....	30.0g
Agar .....	20.0g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of yeasts and molds.

### Saccharococcus Agar

#### Composition per liter:

Agar .....	20.0g
Beef extract .....	5.0g
Sucrose .....	5.0g
Pancreatic digest of casein .....	3.0g
Glucose .....	1.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance *Saccharococcus thermophilus*.

### Saccharolytic Clostridia Medium

#### Composition per liter:

Sodium thioglycolate .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.8g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.2g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
Yeast extract .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CaCl <sub>2</sub> .....	0.01g
Carbohydrate solution .....	100.0mL
Soil extract .....	10.0mL
Trace elements solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Carbohydrate Solution:

##### Composition per 100.0mL:

Glucose or sucrose .....	10.0g
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**Preparation of Carbohydrate Solution:** Add glucose or sucrose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Soil Extract:

**Composition** per 200.0mL:

Garden soil, neutral ..... 100.0g

**Preparation of Soil Extract:** Add garden soil to 100.0mL of tap water. Gently heat and bring to 130°C for 60 min. Cool to 45°C. Filter through Whatman #1 filter paper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Trace Elements Solution:

**Composition** per liter:

Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O ..... 0.05g  
CoNO<sub>3</sub>·6H<sub>2</sub>O ..... 0.05g  
CdSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.05g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.05g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.05g  
MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.05g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except sodium thioglycolate and carbohydrate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Add sodium thioglycolate. Mix thoroughly. Distribute 9.5mL into test tubes that contain inverted Durham tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 0.5mL of sterile carbohydrate solution to each tube. Mix thoroughly.

**Use:** For the isolation of N<sub>2</sub>-fixing, saccharolytic *Clostridium* species.

#### *Salinibacter ruber* Agar (DSMZ Medium 936)

**Composition** per liter:

NaCl ..... 195.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 49.5g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 34.6g  
Agar ..... 20.0g  
KCl ..... 5.0g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.25g  
Yeast extract ..... 1.0g  
NaBr ..... 0.625g  
NaHCO<sub>3</sub> ..... 0.25g  
pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Salinibacter ruber*.

#### *Salinivibrio costicola* Subspecies *vallismortis* Medium (DSMZ Medium 597)

**Composition** per liter:

NaCl ..... 25.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 9.6g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 7.0g  
Glucose ..... 5.0g  
KCl ..... 3.8g

Yeast extract ..... 1.0g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.5g  
K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O ..... 0.4g  
NaHCO<sub>3</sub> solution ..... 100.0mL  
pH 7.0 ± 0.2 at 25°C

#### NaHCO<sub>3</sub> Solution:

**Composition** per 100.0mL:

NaHCO<sub>3</sub> ..... 3.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 100.0mL NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Salinivibrio costicola* subsp. *vallismortis*.

#### *Salmonella* Rapid Test Elective Medium

**Composition** per liter:

Tryptone ..... 10.0g  
Na<sub>2</sub>HPO<sub>4</sub> ..... 9.0g  
Sodium chloride ..... 5.0g  
Casein ..... 5.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.5g  
Malachite Green ..... 0.0025g  
pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the Oxoid *Salmonella* Rapid Test which is for the presumptive detection of motile *Salmonella* in environmental samples.

#### *Salmonella* Rapid Test Elective Medium, 2×

**Composition** per liter:

Tryptone ..... 20.0g  
Na<sub>2</sub>HPO<sub>4</sub> ..... 18.0g  
Sodium chloride ..... 10.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 3.0g  
Casein ..... 10.0g  
Malachite Green ..... 0.005g  
pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** Use as described in the Oxoid *Salmonella* Rapid Test which is for the presumptive detection of motile *Salmonella* in environmental samples.

#### *Salmonella Shigella* Agar (SS Agar)

**Composition** per liter:

Agar ..... 13.5g  
Lactose ..... 10.0g  
Bile salts ..... 8.5g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> ..... 8.5g  
Sodium citrate ..... 8.5g  
Beef extract ..... 5.0g  
Pancreatic digest of casein ..... 2.5g  
Peptic digest of animal tissue ..... 2.5g

Ferric citrate .....	1.0g
Neutral Red .....	0.025g
Brilliant Green .....	0.33mg
pH 7.0 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Do not autoclave. Cool to 45°–50°C. Pour into sterile Petri dishes in 20.0mL volumes. Allow the surface of the plates to dry before inoculation.

**Use:** For the selective isolation and differentiation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella*. This medium is not recommended for the primary isolation of *Shigella* species. Lactose-fermenting bacteria such as *Escherichia coli* or *Klebsiella pneumoniae* appear as small pink or red colonies. Lactose-nonfermenting bacteria—such as *Salmonella* species, *Proteus* species, and *Shigella* species—appear as colorless colonies. Production of H<sub>2</sub>S by *Salmonella* species turns the center of the colonies black.

### Salt Agar

**Composition per liter:**

NaCl .....	58.4g
Agar .....	15.0g
Proteose peptone .....	5.0g
Pancreatic digest of casein .....	5.0g
pH 6.9 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Marinococcus halophilus*.

### Salt Colistin Broth

**Composition per liter:**

NaCl .....	20.0g
Peptone .....	10.0g
Yeast extract .....	3.0g
Colistin solution .....	10.0mL
pH 7.4 ± 0.2 at 25°C	

**Colistin Solution:**

**Composition per 10.0mL:**

Colistin methane sulfonate .....	500,000U
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**Preparation of Colistin Solution:** Add colistin methane sulfonate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except colistin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat until dissolved. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add sterile colistin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of halophilic *Vibrio* species.

### Salt Tolerance Medium

**Composition per liter:**

Beef heart, solids from infusion .....	500.0g
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NaCl .....	65.0g
Tryptose .....	10.0g
pH 7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For testing the salt tolerance of a variety of microorganisms.

### Salt Tolerance Medium, Gilardi

**Composition per liter:**

NaCl .....	65.0g
Pancreatic digest of casein .....	15.0g
Agar .....	15.0g
Papaic digest of soybean meal .....	5.0g
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Do not overheat. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of salt-tolerant, nonfermenting Gram-negative bacteria. For the differentiation of nonfermenting Gram-negative bacteria based on salt tolerance.

### SAP 1 Agar

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
Artificial seawater .....	1.0L
pH 7.2 ± 0.2 at 25°C	

**Artificial Seawater:**

**Composition per liter:**

NaCl .....	24.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.6g
CaCl <sub>2</sub> .....	1.0g
KCl .....	0.7g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to 1.0L of artificial seawater. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### SAP 2 Agar

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
Artificial seawater .....	1.0L
pH 7.2 ± 0.2 at 25°C	

**Artificial Seawater:**

**Composition per liter:**

NaCl .....	24.7g
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MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.6g
CaCl <sub>2</sub> .....	1.0g
KCl .....	0.7g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to 1.0L of artificial seawater. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### *Saprospira grandis* Medium

**Composition** per 1010.0mL:

Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.02g
Seawater, filtered .....	1.0L
Trace elements .....	10.0mL

pH 7.0 ± 0.2 at 25°C

### Trace Elements:

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3mg
H <sub>3</sub> BO <sub>3</sub> .....	0.1mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.1mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.1mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.1mg

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Adjust pH to 7.0. Filter sterilize.

**Use:** For the cultivation of *Saprospira grandis*.

### SB/SW Medium

**Composition** per liter:

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
Sodium crotonate solution .....	50.0mL
NaHCO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Seven vitamin solution .....	10.0mL
Sodium dithionite solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

### Preparation of Trace Elements Solution SL-10:

Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Sodium Crotonate Solution:

**Composition** per 50.0mL:

Sodium crotonate .....	1.1g
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**Preparation of Sodium Crotonate Solution:** Add sodium crotonate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

### NaHCO<sub>3</sub> Solution:

**Composition** per 20.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Seven Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	0.3g
Thiamine-HCl .....	0.2g
Nicotinic acid .....	0.2g
Calcium DL-pantothenate .....	0.1g
Vitamin B <sub>12</sub> .....	0.1g
p-Aminobenzoic acid .....	80.0mg
Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Sodium Dithionite Solution:

**Composition** per 10.0mL:

Sodium dithionite .....	0.2g
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**Preparation of Sodium Dithionite Solution:** Add sodium dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare medium and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except sodium crotonate solution, seven vitamin solution, sodium dithionite solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 910.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile sodium crotonate solution, 20.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of seven vitamin solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and

anaerobically distribute into sterile tubes or flasks. After inoculation, add 0.1mL of sodium dithionite solution per 10.0mL of medium.

**Use:** For the cultivation of *Syntrophobacter buswellii*.

#### SB/SW Medium

##### Composition per liter:

NaCl	1.0g
KCl	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.4g
NH <sub>4</sub> Cl	0.25g
KH <sub>2</sub> PO <sub>4</sub>	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
Resazurin	1.0mg
Sodium crotonate solution	50.0mL
NaHCO <sub>3</sub> solution	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Seven vitamin solution	10.0mL
Sodium dithionite solution	10.0mL
Trace elements solution SL-10	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Sodium Pyruvate Solution:

##### Composition per 50.0mL:

Sodium pyruvate	1.25g
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**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 20.0mL:

NaHCO <sub>3</sub>	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Seven Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	0.3g
Thiamine-HCl	0.2g

Nicotinic acid	0.2g
Calcium DL-pantothenate	0.1g
Vitamin B <sub>12</sub>	0.1g
p-Aminobenzoic acid	80.0mg
Biotin	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Sodium Dithionite Solution:

##### Composition per 10.0mL:

Sodium dithionite	0.2g
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**Preparation of Sodium Dithionite Solution:** Add sodium dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare medium and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except sodium pyruvate solution, seven vitamin solution, sodium dithionite solution, NaHCO<sub>3</sub> solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 910.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile sodium pyruvate solution, 20.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of seven vitamin solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks. After inoculation, add 0.1mL of sodium dithionite solution per 10.0mL of medium.

**Use:** For the cultivation of *Syntrophobacter wolnii*.

#### SC Agar

##### Composition per liter:

Agar	15.0g
Papaic digest of soybean meal	8.0g
Cornmeal (solids from infusion)	2.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
KH <sub>2</sub> PO <sub>4</sub>	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Hemin solution	15.0mL
Bovine serum albumin, fraction V	10.0mL
L-Cysteine·H <sub>2</sub> O solution	10.0mL
Glucose solution	1.0mL

pH 6.6 ± 0.2 at 25°C

#### Hemin Solution:

##### Composition per 100.0mL:

Hemin	0.1g
NaOH (0.05N solution)	100.0mL

**Preparation of Hemin Solution:** Add hemin to NaOH solution. Mix thoroughly.

#### Bovine Serum Albumin, Fraction V Solution:

##### Composition per 10.0mL:

Bovine serum albumin, fraction V	2.0g
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**Preparation of Bovine Serum Albumin, Fraction V Solution:** Add bovine serum albumin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### L-Cysteine·H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

L-Cysteine·H <sub>2</sub> O	1.0g
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**Preparation of L-Cysteine·H<sub>2</sub>O Solution:** Add L-cysteine·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Glucose Solution:****Composition per 10.0mL:**

D-Glucose ..... 5.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except bovine serum albumin, L-cysteine-H<sub>2</sub>O solution, and glucose solution, to distilled/deionized water and bring volume to 979.0mL. Mix thoroughly. Adjust pH to 6.6 with NaOH. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile bovine serum albumin, 10.0mL of sterile L-cysteine-H<sub>2</sub>O solution, and 1.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Clavibacter xyli*.

**SC Medium****Composition per 1021.0mL:**

Agar ..... 15.0g  
 Papaic digest of soybean meal ..... 8.0g  
 Cornmeal, solids from infusion ..... 2.0g  
 Tween 80 ..... 1.0g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
 Hemin chloride solution ..... 15.0mL  
 Bovine serum albumin solution ..... 10.0mL  
 L-Cysteine solution ..... 10.0mL  
 Glucose solution ..... 1.0mL

pH 6.6 at 25°C

**Hemin Chloride Solution:****Composition per 100.0mL:**

Hemin chloride ..... 0.1g  
 NaOH (0.05N solution) ..... 100.0mL

**Preparation of Hemin Chloride Solution:** Add hemin chloride to 100.0mL of NaOH solution. Mix thoroughly.

**Bovine Serum Albumin Solution:****Composition per 10.0mL:**

Bovine serum albumin ..... 2.0g

**Preparation of Bovine Serum Albumin Solution:**

Add bovine serum albumin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**L-Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine, free base ..... 1.0g

**Preparation of L-Cysteine Solution:** Add L-cysteine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Glucose Solution:****Composition per 10.0mL:**

Glucose ..... 5.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except bovine serum albumin solution, L-cysteine solution, and glucose solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling.

Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile bovine serum albumin solution, 10.0mL of sterile L-cysteine solution, and 1.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of coryneform bacteria that cause ratoon stunting disease of sugarcane.

**SCY Medium  
(Maintenance SCY Medium)****Composition per liter:**

Solution A ..... 1.0L  
 Solution B ..... 200.0mL

**Solution A:****Composition per liter:**

Agar ..... 10.0g  
 Pancreatic digest of casein ..... 0.92g  
 NaCl ..... 0.05g  
 Papaic digest of soybean meal ..... 0.03g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 0.025g

pH 7.0 ± 0.2 at 25°C

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Solution B:****Composition per 200.0mL:**

Sucrose ..... 2.0g  
 Yeast extract ..... 0.5g  
 Thiamine ..... 0.8mg  
 Vitamin B<sub>12</sub> ..... 0.02mg

pH 8.5 ± 0.2 at 25°C

**Preparation of Solution B:** Add components to slightly alkaline tap water, pH 8.5, and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Inoculate bacteria onto prepared slants of solution A. After inoculation of tubes, aseptically add 2.0mL of sterile solution B on top of each slant.

**Use:** For the cultivation and maintenance of iron bacteria. For the cultivation and maintenance of *Haliscomenobacter hydrossis*.

**Seawater Agar  
(SWA)****Composition per liter:**

Agar ..... 15.0g  
 Peptone ..... 5.0g  
 Yeast extract ..... 5.0g  
 Beef extract ..... 3.0g  
 Seawater, synthetic ..... 1.0L

pH 7.5 ± 0.2 at 25°C

**Seawater, Synthetic:****Composition per liter:**

NaCl ..... 27.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 7.0g  
 Tris(hydroxymethyl)aminomethane buffer ..... 2.0g  
 KCl ..... 0.6g  
 CaCl<sub>2</sub> ..... 0.3g

**Preparation of Seawater, Synthetic:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Distribute into



tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of halophilic microorganisms, such as *Pseudomonas* species and *Vibrio* species, from fish.

#### Seawater Agar

##### Composition per 1.0L:

Agar .....	20.0g
Beef extract .....	10.0g
Peptone .....	10.0g
Seawater .....	750.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective isolation and cultivation of *Planococcus* species.

#### Seawater Agar (SWA)

##### Composition per liter:

Agar .....	15.0g
Peptone .....	5.0g
Yeast extract .....	5.0g
Beef extract .....	3.0g
Seawater, synthetic .....	1.0L

pH 7.5 ± 0.2 at 25°C

#### Seawater, Synthetic:

##### Composition per liter:

NaCl .....	24.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.3g
KCl .....	0.7g
CaCl <sub>2</sub> .....	0.1g

**Preparation of Seawater, Synthetic:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5.

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of halophilic *Vibrio* species from fish.

#### Seawater Agar

##### Composition per liter:

Agar .....	20.0g
Beef extract .....	10.0g
Peptone .....	10.0g
Seawater .....	750.0mL

#### Artificial Seawater:

##### Composition per liter:

NaCl .....	28.13g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.5g
MgCl <sub>2</sub> .....	2.55g
CaCl <sub>2</sub> .....	1.2g
KCl .....	0.77g
NaHCO <sub>3</sub> .....	0.11g

pH 7.3 ± 0.2 at 25°C

**Preparation of Artificial Seawater:** Natural seawater is stored in the dark for at least 3 weeks to “age.” If natural

seawater is not available, use artificial seawater. To prepare artificial seawater, add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add beef extract and peptone to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Adjust pH to 7.8. Gently heat and bring to boiling. Boil for 10.0 min. Add 750.0mL of natural or artificial seawater. Mix thoroughly. Adjust pH to 7.3. Add 20.0g of agar. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alteromonas rubra*, *Brevibacterium stationis*, *Chromohalobacter marismortui*, *Flectobacillus marinus*, *Marinococcus albus*, *Marinococcus halophilus*, *Pasteurella piscicida*, *Photobacterium phosphoreum*, *Planococcus citreus*, *Planococcus kocurii*, *Vibrio adaptatus*, *Vibrio campbellii*, *Vibrio costicola*, *Vibrio harveyi*, *Vibrio mediterranei*, *Vibrio natriegens*, and other *Vibrio* species.

#### Seawater Agar with Fetal Calf Serum

##### Composition per liter:

Agar .....	20.0g
Beef extract .....	10.0g
Peptone .....	10.0g
Seawater .....	750.0mL
Fetal calf serum .....	100.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except fetal calf serum, to tap water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile fetal calf serum warmed to 50°–55°C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Aeromonas* species and *Vibrio salmonicida*.

#### Seawater Agar with Horse Blood

##### Composition per liter:

Agar .....	20.0g
Beef extract .....	10.0g
Peptone .....	10.0g
Seawater .....	750.0mL
Horse blood .....	100.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except horse blood, to tap water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile horse blood warmed to 50°–55°C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Aeromonas* species.

#### Seawater Agar Medium

##### Composition per liter:

Agar .....	15.0g
Beef extract .....	10.0g
Peptone .....	10.0g
Seawater, aged .....	750.0mL

pH 7.2–7.3 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of marine *Flavobacterium* species.

#### Seawater Agar Modified (DSMZ Medium 917)

**Composition** per liter:

NaCl .....	17.7g
Agar .....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.46g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.4g
Peptone .....	2.5g
Hexadecane .....	2.0g
Yeast extract .....	1.5g
KCl .....	0.48g
Calcium chloride solution .....	10.0mL
pH 7.2 ± 0.2 at 25°C	

#### Calcium Chloride Solution:

**Composition** per 10.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.98g
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**Preparation of Calcium Chloride Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except agar and calcium chloride solution, to distilled/deionized water and bring volume to 990.0mL. Adjust pH to 7.2. Mix thoroughly. Add 15.0g agar. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL sterile calcium chloride solution. Mix thoroughly. Pour into sterile Petri dishes or distribute to sterile tubes.

**Use:** For the cultivation and maintenance of *Muricauda rustringensis*.

#### Seawater Agar with 1% Serum

**Composition** per liter:

Agar .....	12.0g
Seawater .....	990.0mL
Horse serum .....	10.0mL

**Preparation of Medium:** Add agar to 990.0mL of seawater. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile horse serum. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Basipetospora halophila*, *Halosphaeria retorquens*, *Thraustochytrium striatum*, and *Lagenidium callinectes*.

#### Seawater Basal Medium

**Composition** per liter:

NH <sub>4</sub> Cl .....	10.0g
Lactate .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	75.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.0mg
Tris (hydroxymethyl) amino methane .....	50.0mM
Artificial seawater .....	500.0mL

#### Artificial Seawater:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	24.65g
NaCl .....	23.37g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.94g
KCl .....	1.49g
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of marine bacteria.

#### Seawater Complete Medium

**Composition** per liter:

Pancreatic digest of casein .....	5.0g
Yeast extract .....	3.0g
Seawater .....	750.0mL
Glycerol .....	3.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Vibrio fischeri*.

#### Seawater Lemco Agar

**Composition** per liter:

Agar .....	15.0g
Beef extract .....	10.0g
Peptone .....	10.0g
Seawater, filtered aged .....	750.0mL
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.8. Gently heat and bring to boiling. Boil for 3–5 min. Filter through Whatman filter paper. Adjust pH to 7.3. Add agar. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Halococcus nondenitrificans*.

#### Seawater Medium

**Composition** per liter:

Agar .....	15.0g
Peptone .....	5.0g
Beef extract .....	2.0g
KNO <sub>3</sub> .....	0.5g
Seawater, aged .....	1.0L
pH 7.8 ± 0.2 at 25°C	

**Preparation of Medium:** Combine components. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of halophilic bacteria.

#### Seawater 802 Medium

**Composition** per liter:

Solution A .....	500.0mL
Solution B .....	500.0mL

#### Solution A:

**Composition** per 500.0mL:

NaCl .....	27.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.38g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.78g
KCl .....	0.72g
NaHCO <sub>3</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.4g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Filter sterilize.

#### **Solution B:**

**Composition per liter:**

Rye grass cerophyll ..... 5.0g

**Preparation of Solution B:** Add cerophyll to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to a boil. Boil for 5 min. Filter through Whatman #1 filter paper. Add 0.5g of Na<sub>2</sub>HPO<sub>4</sub>. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Distribute 10.0mL volumes into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Source:** Cerophyll can be obtained from Ward's Natural Science Establishment, Inc. Dairy Goat Nutrition distributes Grass Media Culture, which is equivalent. Cereal Leaf Product from Sigma Chemical is similar to cerophyll.

**Preparation of Medium:** Aseptically add 500.0mL of sterile solution A and 500.0mL of sterile solution B. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Amastigomonas bermudensis*, *Ancyromonas sigmoides*, *Bodo curvifilus*, *Bodo saliens*, *Cafeteria roenbergensis*, *Cafeteria minuta*, *Ciliophrys infusionum*, *Cruzella marina*, *Glauconema bermudense*, *Helkesimastix faecicola*, *Jakoba libera*, *Massisteria marina*, *Monosiga brevicollis*, *Percolomonas cosmopolitus*, *Peridomonas danica*, and *Trimyema shoalsia*.

**Use:** For the cultivation of *Euplotes harpa*.

#### **Seawater Nitrosomonas Medium**

**Composition per 1003.3mL:**

HEPES (N-[2-Hydroxyethyl]piperazine-N'-2-ethanesulfonic acid) buffer ..... 4.76g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 20.0mg  
K<sub>2</sub>HPO<sub>4</sub> ..... 15.0mg  
Artificial seawater ..... 1.0L  
K<sub>2</sub>CO<sub>3</sub> (5% solution) ..... 2.0mL  
Trace elements solution ..... 1.0mL  
Phenol Red (0.04% solution) ..... 0.3mL  
pH 7.8 ± 0.2 at 25°C

#### **Trace Elements Solution:**

**Composition per liter:**

EDTA ..... 2.06g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.54g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.2g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.1g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 20.0mg  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 2.0mg  
HCl, concentrated ..... 83.0mL

**Preparation of Trace Elements:** Add HCl and EDTA to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Add remaining components. Mix thoroughly.

#### **Artificial Seawater:**

**Composition per liter:**

NaCl ..... 27.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 6.78g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.38g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.4g

KCl ..... 0.72g  
NaHCO<sub>3</sub> ..... 0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Adjust pH to 7.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Nitrosomonas cryotolerans*.

#### **Seawater Nutrient Agar (SNA) (ATCC Medium 2205)**

**Composition per liter:**

Nutrient agar, 2× ..... 500.0mL  
Artificial seawater, 2× ..... 500.0mL  
pH 7.3 ± 0.2 at 25°C

**Nutrient Agar, 2×:**

**Composition per 500.0mL:**

Agar ..... 15.0g  
Peptone ..... 5.0g  
NaCl ..... 5.0g  
Yeast extract ..... 2.0g  
Beef extract ..... 1.0g

**Preparation of Nutrient Agar, 2×:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Seawater, Synthetic 2×:**

**Composition per 500.0mL:**

NaCl ..... 24.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 7.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.3g  
KCl ..... 0.7g  
CaCl<sub>2</sub> ..... 0.1g

**Preparation of Seawater, Synthetic:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 7.5. Filter sterilize.

**Preparation of Medium:** Warm synthetic seawater to 50°C. Aseptically combine sterile nutrient agar and sterile synthetic seawater. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of marine bacteria.

#### **Seawater with Serum**

**Composition per liter:**

Agar ..... 10.0g  
Seawater ..... 1.0L  
Bovine serum, sterile ..... 100.0mL

**Preparation of Medium:** Add agar to 1.0L of seawater. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile liquid beef serum. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Basipetospora halophila*, *Halosphaeria retorquens*, *Lagenidium callinectes*, and *Thraustochytrium striatum*.

#### **Seawater Spirillum Medium**

**Composition per liter:**

Calcium lactate ..... 10.0g

Peptone.....	5.0g
Beef extract.....	3.0g
Yeast extract.....	3.0g
Seawater.....	750.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 20 min at 10 psi pressure–115°C. A precipitate will form during autoclaving.

**Use:** For the cultivation of marine *Spirillum* species.

#### Seawater Yeast Extract Agar

**Composition** per liter:

Marine salts mix.....	37.9g
Agar.....	15.0g
Proteose peptone.....	10.0g
Yeast extract.....	3.0g
pH 7.2–7.4 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Alteromonas* species, *Caulobacter halobacteroides*, *Caulobacter maris*, *Cytophaga marinoflava*, and *Cytophaga salmonicolor*.

#### Seawater Yeast Extract Broth, Modified

**Composition** per liter:

NaCl.....	23.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.9g
Peptone.....	1.0g
Yeast extract.....	1.0g
KCl.....	0.75g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Proteus* species and *Vibrio* species.

#### Seawater Yeast Extract Peptone Medium

**Composition** per liter:

Agar.....	15.0g
Peptone.....	5.0g
Yeast extract.....	3.0g
Seawater, aged and filtered.....	750.0mL
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.8. Gently heat and bring to boiling. Continue boiling for 3–5 min. Filter through Whatman filter paper. Adjust pH to 7.3. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Planococcus kocurii*.

#### Seawater Yeast Peptone Agar (DSMZ Medium 243)

**Composition** per liter:

Agar.....	12.0g
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Peptone.....	5.0g
Yeast extract.....	3.0g
Filtered, seawater.....	750.0mL
pH 7.3 ± 0.2 at 25°C	

#### Artificial Seawater:

NaCl.....	28.13g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	4.8g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.6g
KCl.....	0.77g
NaHCO <sub>3</sub> .....	0.11g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add peptone and yeast extract to 250.0mL distilled/deionized water. Mix thoroughly. Adjust pH to 7.8. Boil for 5 min. Filter and readjust the pH to 7.3. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45–50°C. Add 750.0mL filter sterilized seawater that has been heated to 50°C. (Note: Natural seawater is stored in the dark for at least 3 weeks to age. If natural seawater is not available use artificial seawater.) Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Zobellia uliginosa*=*Cellulophaga uliginosa*=*Cytophaga uliginosa*, *Marinobacterium jannaschii*=*Oceanospirillum jannaschii*, *Vibrio harveyi*=*Lucibacterium harveyi*, *Halomonas* sp., and *Pseudoalteromonas espejiana*=*Alteromonas espejiana*.

#### Seawater Yeast Peptone Medium (DSMZ Medium 949)

**Composition** per liter:

Peptone.....	5.0g
Yeast extract.....	3.0g
Seawater, filtered and aged.....	750.0mL
pH 7.3 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Shewanella algae*.

#### *Selenomonas acidaminophila* Medium

**Composition** per liter:

Disodium β-glycerophosphate.....	19.0g
Beef extract.....	5.0g
Lactose.....	5.0g
Papaic digest of soybean meal.....	5.0g
Sodium glutamate.....	3.4g
Pancreatic digest of casein.....	2.5g
Peptic digest of animal tissue.....	2.5g
Yeast extract.....	2.5g
Ascorbic acid.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
pH 7.15 ± 0.05 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Selenomonas acidaminophila*.

***Selenomonas ruminantium* Medium****Composition** per liter:

Pancreatic digest of casein	5.0g
Na <sub>2</sub> CO <sub>3</sub>	4.0g
Sodium acetate	4.0g
Yeast extract	2.0g
Glucose	1.0g
KH <sub>2</sub> PO <sub>4</sub>	1.0g
L-Cysteine-HCl	0.5g
Resazurin	1.0mg
n-Valeric acid	0.1mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Prepare and dispense medium under 100% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% CO<sub>2</sub>. Adjust pH to 7.0. Anaerobically distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Selenomonas ruminantium* and *Selenomonas* species.

***Selenomonas* Selective Medium (SS Medium)****Composition** per 100.0mL:

Pancreatic digest of casein	0.5g
Mannitol	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
Sodium acetate	0.1g
Yeast extract	0.1g
L-Cysteine-HCl	0.08g
Mineral solution S	4.0mL
Sodium carbonate (8% solution)	2.5mL
n-Valeric acid	0.05mL

pH 5.9–6.1 at 25°C

**Mineral Solution S:****Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub>	12.0g
NaCl	12.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	6.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.6g

**Preparation of Mineral Solution S:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Selenomonas* species.

**Seven-Hour Fecal Coliform Agar (Seven-Hour FC Agar)****(m-Seven-Hour Fecal Coliform Agar)****Composition** per liter:

Agar	15.0g
Lactose	10.0g
NaCl	7.5g
D-Mannitol	5.0g
Proteose peptone No. 3	5.0g
Yeast extract	3.0g
Bromcresol Purple	0.35g
Phenol Red	0.3g

Sodium lauryl sulfate	0.2g
Sodium deoxycholate	0.1g

pH 7.3 ± 0.1 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to 55°–60°C. Adjust pH to 7.3 with 0.1N NaOH. Cool to 45°–50°C. Pour into sterile Petri dishes with tight-fitting lids in 5.0mL volumes. Store at 2°–10°C.

**Use:** For the rapid estimation of the bacteriological quality of water using the membrane filter method.

**SF1 Medium****Composition** per liter:

NaCl	120.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	7.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	6.0g
KCl	3.8g
Pancreatic digest of casein	2.0g
Yeast extract	2.0g
NH <sub>4</sub> Cl	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.5g
L-Cysteine-HCl	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.4g
Resazurin	1.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	75.0μg
Na <sub>2</sub> CO <sub>3</sub> solution	20.0mL
Trimethylamine-HCl solution	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Trace elements solution SL-10	1.0mL
NaOH (10M solution)	0.6mL

pH 7.3 ± 0.2 at 25°C

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 20.0mL:

Na <sub>2</sub> CO <sub>3</sub>	10.0mg
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub>.

**Trimethylamine-HCl Solution:****Composition** per 20.0mL:

Trimethylamine-HCl	10.0mg
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**Preparation of Trimethylamine-HCl Solution:**

Add trimethylamine-HCl to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub>.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	10.0mg
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Store under N<sub>2</sub>.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

# **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except L-cysteine-HCl, NaOH, Na<sub>2</sub>CO<sub>3</sub> solution, trimethylamine-HCl solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add L-cysteine-HCl and NaOH while continuing to sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 6.7. Anaerobically distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile trimethylamine-HCl solution, 20.0mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution per 950.0mL of medium. Check that final pH is 6.7.

**Use:** For the cultivation and maintenance of *Methanohalophilus* species.

## **SG Agar**

### **Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	15.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

## **SIM Medium**

### **Composition per liter:**

Peptone.....	30.0g
Agar .....	3.0g
Beef extract .....	3.0g
Peptonized iron .....	0.2g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.025g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 15.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in an upright position.

**Use:** For the differentiation of members of the Enterobacteriaceae based on H<sub>2</sub>S production, indole production, and motility.

## **SIM Medium**

### **Composition per liter:**

Pancreatic digest of casein .....	20.0g
Peptic digest of animal tissue.....	6.1g
Agar .....	3.5g

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	0.2g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 15.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in an upright position.

**Use:** For the differentiation of members of the Enterobacteriaceae based on H<sub>2</sub>S production, indole production, and motility.

## **Simmons' Citrate Agar (Citrate Agar)**

### **Composition per liter:**

Agar .....	15.0g
NaCl .....	5.0g
Sodium citrate .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Bromothymol Blue .....	0.08g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the differentiation of Gram-negative bacteria on the basis of citrate utilization. Bacteria that can utilize citrate as sole carbon source turn the medium blue.

## **Simonsiella Agar (LMG Medium 31)**

### **Composition per liter:**

Tryptone .....	17.0g
Agar .....	15.0g
NaCl .....	5.0g
Yeast extract .....	4.0g
Soya peptone .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Bovine serum.....	100.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except bovine serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL sterile bovine serum. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Simonsiella* spp.

## **Simonsiella Agar**

### **Composition per liter:**

Pancreatic digest of casein .....	17.0g
Agar .....	15.0g
NaCl .....	5.0g
Yeast extract .....	4.0g
Papaic digest of soybean meal .....	3.0g

K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Horse serum .....	100.0mL

**Preparation of Medium:** Add components, except horse serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile horse serum warmed to 50°–55°C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Simonsiella muelleri* and *Simonsiella steedae*.

### Singh's Medium, Modified

**Composition per liter:**

NaCl .....	8.75g
Lactalbumin hydrolysate.....	8.13g
Yeast extract .....	6.25g
D-Glucose .....	5.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KCl .....	0.25g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	0.25g
NaHCO <sub>3</sub> .....	0.15g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.13g
Phenol Red .....	0.01g
Fetal bovine serum (heat inactivated at 56°C, 30 min).....	200.0mL
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with NaOH if necessary. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma* species.

### SJ Agar

**Composition per liter:**

Agar .....	15.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KCl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaNO <sub>3</sub> .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Glucose solution .....	100.0mL
pH 7.2 ± 0.2 at 25°C	

### Glucose Solution:

**Composition per 100.0mL:**

D-Glucose .....	1.0g
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**Preparation of Glucose Solution:** Add D-glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### Skim Milk Acetate Medium

**Composition per liter:**

Agar .....	15.0g
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Skim milk powder .....	5.0g
Yeast extract .....	0.5g
Sodium acetate .....	0.2g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Cytophaga johnsonae*.

### Skim Milk Agar

(Milk Agar)

(ATCC Medium 377)

**Composition per liter:**

Agar .....	15.0g
Skim milk .....	8.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Herpetosiphon aurantiacus*.

### SL Medium

(DSMZ Medium 959)

**Composition per liter:**

NaCl .....	15.0g
PIPES .....	3.4g
Na-acetate·3H <sub>2</sub> O .....	2.72g
Yeast extract .....	2.0g
Trypticase .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.35g
KH <sub>2</sub> PO <sub>4</sub> .....	0.35g
KCl .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Resazurin .....	0.5mg
Maltose solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
pH 47.5 ± 0.2 at 25°C	

### Maltose Solution:

**Composition per 100.0mL:**

Maltose .....	3.5g
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**Preparation of Maltose Solution:** Add maltose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 50.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	5.0g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 50.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add components, except maltose solution and Na<sub>2</sub>S·9H<sub>2</sub> solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Sparge with N<sub>2</sub>. Adjust pH to 7.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 50.0mL sterile maltose solution. Mix thoroughly.

Aseptically and anaerobically distribute into sterile tubes or flasks under N<sub>2</sub>. The final pH should be 7.5.

**Use:** For the cultivation of *Petrotoga olearia* and *Petrotoga sibirica*.

#### Slanetz and Bartley Medium

**Composition** per liter:

Tryptose .....	20.0g
Agar .....	10.0g
Yeast extract .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> 2H <sub>2</sub> O .....	4.0g
Glucose .....	2.0g
NaN <sub>3</sub> .....	0.4g
Tetrazolium chloride .....	0.1g

pH 7.2 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the detection and enumeration of enterococci by the membrane filter method.

#### Sludge Medium for Methanobacteria

**Composition** per liter:

NaHCO <sub>3</sub> .....	4.0g
Sodium formate .....	2.0g
Sodium acetate .....	1.0g
Yeast extract .....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Sludge fluid .....	50.0mL
Fatty acid mixture .....	20.0mL
Trace elements SL-6 .....	1.0mL

pH 6.7–7.0 at 25°C

#### Sludge Fluid:

**Composition** per 100.0mL:

Yeast extract .....	0.4g
Sludge .....	100.0mL

**Preparation of Sludge Fluid:** Add yeast extract to a concentration of 0.4% to sludge taken from an anaerobic digester. Gas with 100% N<sub>2</sub> for 5 min. Incubate at 37°C for 24 hr. Centrifuge at 13,000 × g. Remove the supernatant fluid. Gas with 100% N<sub>2</sub> for 5 min. Autoclave for 15 min at 15 psi pressure–121°C. Store at 25°C protected from light.

#### Fatty Acid Mixture:

**Composition** per 20.0mL:

α-Methylbutyric acid .....	0.5g
Isobutyric acid .....	0.5g
Isovaleric acid .....	0.5g
Valeric acid .....	0.5g

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Adjust pH to 7.5 with concentrated NaOH.

#### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.7–7.0. Distribute anaerobically into tubes or bottles with aluminum seals. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation of *Methanobacterium uliginosum* and *Methanobrevibacter ruminantium*.

#### Sludge Medium for Methanobacteria, pH 7.9

**Composition** per liter:

NaHCO <sub>3</sub> .....	4.0g
Sodium formate .....	2.0g
Sodium acetate .....	1.0g
Yeast extract .....	1.0g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4g
NaCl .....	0.4g
NH <sub>4</sub> Cl .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Sludge fluid .....	50.0mL
Fatty acid mixture .....	20.0mL
Trace elements SL-6 .....	1.0mL

pH 7.9 ± 0.2 at 25°C

#### Sludge Fluid:

**Composition** per 100.0mL:

Yeast extract .....	0.4g
Sludge .....	100.0mL

**Preparation of Sludge Fluid:** Add yeast extract to a concentration of 0.4% to sludge taken from an anaerobic digester. Gas with 100% N<sub>2</sub> for 5 min. Incubate at 37°C for 24 hr. Centrifuge at 13,000 × g. Remove the supernatant fluid. Gas with 100% N<sub>2</sub> for 5 min. Autoclave for 15 min at 15 psi pressure–121°C. Store at 25°C protected from light.

#### Fatty Acid Mixture:

**Composition** per 20.0mL:

α-Methylbutyric acid .....	0.5g
Isobutyric acid .....	0.5g
Isovaleric acid .....	0.5g
Valeric acid .....	0.5g

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Adjust pH to 7.5 with concentrated NaOH.

#### Trace Elements Solution SL-6:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g



ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 7.9. Distribute anaerobically into tubes or bottles with aluminum seals. Autoclave for 15 min at 15 psi pressure–121°C with fast exhaust.

**Use:** For the cultivation of *Methanobacterium alcaliphilum* and *Methanobacterium thermoacalip*.

### SM Basal Salts Medium

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	4.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Trace metals solution .....	5.0mL

pH 6.0 ± 0.2 at 25°C

### Trace Metals Solution:

**Composition per liter:**

Ethylenediamine tetraacetate .....	50.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	22.0g
CaCl <sub>2</sub> .....	5.54g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.99g
CoCl <sub>2</sub> ·H <sub>2</sub> O .....	1.61g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	1.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus delicatus*.

### SM Medium

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> .....	4.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution .....	100.0mL
Trace metals solution .....	5.0mL

pH 7.5 ± 0.2 at 25°C

### Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution .....	10.0g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add 10.0g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

### Trace Metals Solution:

**Composition per liter:**

Ethylenediaminetetraacetic acid .....	50.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	22.0g
CaCl <sub>2</sub> .....	5.54g

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.99g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.61g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·H <sub>2</sub> O .....	1.10g

**Preparation of Trace Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 using KOH. Filter sterilize.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and trace metals solution, to distilled/deionized water and bring volume to 895.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and 5.0mL of sterile trace metals solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus thioparus*.

### SMC Medium

**Composition per liter:**

Sorbitol .....	70.0g
Pancreatic digest of casein .....	17.0g
NaCl .....	5.0g
Beef extract .....	3.0g
Yeast extract .....	3.0g
Beef heart, solids from infusion .....	2.0g
Horse serum .....	200.0mL
Yeast extract solution .....	100.0mL
Phenol Red solution .....	20.0mL
Sucrose solution .....	20.0mL
L-Arginine·HCl solution .....	10.0mL
Fructose solution .....	2.0mL
Glucose solution .....	2.0mL

pH 7.5 ± 0.2 at 25°C

### Yeast Extract Solution:

**Composition per 100.0mL:**

Yeast extract .....	25.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

### Phenol Red Solution:

**Composition per 100.0mL:**

Phenol Red .....	0.01g
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**Preparation of Phenol Red Solution:** Add Phenol Red to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

### Sucrose Solution:

**Composition per 20.0mL:**

Sucrose .....	10.0g
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**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

### L-Arginine·HCl Solution:

**Composition per 10.0mL:**

L-Arginine·HCl .....	4.2g
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**Preparation of L-Arginine·HCl Solution:** Add L-arginine·HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Fructose Solution:****Composition** per 10.0mL:

Fructose ..... 5.0g

**Preparation of Fructose Solution:** Add fructose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Glucose Solution:****Composition** per 10.0mL:

Glucose ..... 5.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add components, except horse serum, yeast extract solution, Phenol Red solution, sucrose solution, L-arginine-HCl solution, fructose solution, and glucose solution, to distilled/deionized water and bring volume to 646.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 200.0mL of sterile horse serum, 100.0mL of sterile yeast extract solution, 20.0mL of sterile Phenol Red solution, 20.0mL of sterile sucrose solution, 10.0mL of sterile L-arginine-HCl solution, 2.0mL of sterile fructose solution, and 2.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma citri*.

**SMC, Modified****Composition** per liter:

Sorbitol ..... 70.0g  
 Pancreatic digest of casein ..... 17.0g  
 NaCl ..... 5.0g  
 Beef extract ..... 3.0g  
 Yeast extract ..... 3.0g  
 Beef heart, solids from infusion ..... 2.0g  
 Solution 1 ..... 100.0mL  
 Solution 3 ..... 20.0mL  
 Solution 2 ..... 10.0mL  
 NaOH (1N solution) ..... 6.0mL

pH 7.7–7.8 at 25°C

**Solution 1:****Composition** per 100.0mL:

Sucrose ..... 10.0g  
 Yeast extract ..... 2.0g  
 Fructose ..... 1.0g  
 Glucose ..... 1.0g  
 Phenol Red ..... 0.02g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution 2:****Composition** per 10.0mL:

Bovine serum albumin, fraction V ..... 0.1g

**Preparation of Solution 2:** Add bovine serum albumin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Solution 3:****Composition** per 20.0mL:

Horse serum ..... 20.0mL

**Preparation of Solution 3:** Inactivate horse serum at 56°C for 30 min. Filter sterilize.

**Preparation of Medium:** Add components, except solution 1, solution 2, and solution 3, to distilled/deionized water and bring volume to 870.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile solution 1, 20.0mL of sterile solution 3, and 10.0mL of sterile solution 2. Mix thoroughly. Adjust pH to 7.7–7.8. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma citri*.

**SME Agar  
(ATCC Medium 2345)****Composition** per 1010.0mL:

NaCl ..... 27.7g  
 Agar ..... 18.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 7.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.5g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.5g  
 KCl ..... 0.65g  
 NaBr ..... 0.1g  
 H<sub>3</sub>BO<sub>3</sub> ..... 30.0mg  
 SrCl<sub>2</sub>·6H<sub>2</sub>O ..... 15.0mg  
 KI ..... 0.05mg  
 Tris-HCl buffer (1.0M solution, pH 7.0) ..... 25.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Aquifex pyrophilus*.

**SME Medium, Modified****Composition** per 1010.0mL:

NaCl ..... 30.0g  
 MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 7.0g  
 NaHCO<sub>3</sub> ..... 2.0g  
 KCl ..... 0.65g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.5g  
 K<sub>2</sub>HPO<sub>4</sub> ..... 0.15g  
 NH<sub>4</sub>Cl ..... 0.15g  
 NaBr ..... 0.1g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.5mg  
 Trace elements solution ..... 10.0mL

pH 6.5 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
 (NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> ..... 2.0g  
 Nitrilotriacetic acid ..... 1.5g  
 NaCl ..... 1.0g  
 MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.5g  
 CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.025g  
 KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.02g  
 CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
 CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g  
 Na<sub>2</sub>WO<sub>4</sub> ..... 10.0mg

Na <sub>2</sub> SeO <sub>4</sub> .....	10.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 20 min. Adjust pH to 6.5–6.8 with H<sub>2</sub>SO<sub>4</sub>. Distribute 10.0mL volumes into 120.0mL serum bottles while gassing under 100% CO<sub>2</sub>. Stopper each serum bottle tightly. Exchange the gas phase in each serum bottle with 79.75% H<sub>2</sub> + 19.75% CO<sub>2</sub> + 0.5% O<sub>2</sub> and bring pressure to 300KPa. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Aquifex pyrophilus*.

### Soil Extract

**Composition per 200.0mL:**

African Violet soil .....	77.0g
Na <sub>2</sub> CO <sub>3</sub> .....	0.2g

**Preparation of Medium:** Add components to 200.0mL of distilled/deionized water. Mix thoroughly. Autoclave for 60 min at 15 psi pressure–121°C. Filter through paper and reserve filtrate.

**Use:** Used as a growth factor additive for the cultivation of soil bacteria and fungi.

### Soil Extract Agar

**Composition per liter:**

Agar .....	20.0g
Glucose .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Peptone .....	1.0g
Yeast extract .....	1.0g
Soil extract .....	400.0mL
Cycloheximide solution .....	10.0mL
pH 6.6 ± 0.2 at 25°C	

**Soil Extract:**

**Composition per liter:**

Garden soil, neutral .....	1.0Kg
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**Preparation of Soil Extract:** Add garden soil to 1.0L of tap water. Autoclave for 20 min at 15 psi pressure–121°C. Filter through Whatman filter paper. Bring volume to 1.0L with tap water.

**Cycloheximide Solution:**

**Composition per 10.0mL:**

Cycloheximide .....	0.04g
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**Preparation of Cycloheximide Solution:** Add cycloheximide to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cycloheximide solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile cycloheximide solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of *Arthrobacter* species.

### Soil Extract Agar

**Composition per liter:**

Soil .....	500.0g
Agar .....	15.0g
Glucose .....	2.0g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g

**Preparation of Medium:** Add 500.0g of garden soil to 1.0L of tap water. Autoclave for 3 hr at 15 psi pressure–121°C. Filter through Whatman #2 filter paper. Add remaining components to filtrate. Bring volume to 1.0L with tap water. Gently heat and bring to boiling. Distribute into tubes in 7.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position.

**Use:** For the cultivation and identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Bacillus* species based on the formation of typical conidia.

### Soil Extract Agar

**Composition per liter:**

Agar .....	15.0g
Soil extract .....	1.0L
pH 6.8 ± 0.2 at 25°C	

**Soil Extract:**

**Composition per liter:**

Soil .....	400.0g
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**Preparation of Soil Extract:** Air dry garden soil with a high content of organic matter and pass through a sieve. Weigh out 400.0g and add to 960.0mL of tap water. Autoclave for 60 min at 15 psi pressure–121°C. Cool to room temperature and allow soil to settle out. Decant supernatant solution. Filter through paper. Bring volume to 1.0L with distilled/deionized water.

**Preparation of Medium:** Add agar to 1.0L soil extract. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Aureobacterium flavescens* and *Bacillus* species.

### Soil Extract Glucose Yeast Extract Agar

**Composition per liter:**

Agar .....	15.0g
Glucose .....	2.0g
Yeast extract .....	1.0g
Soil extract .....	250.0mL
pH 6.8 ± 0.2 at 25°C	

**Soil Extract:**

**Composition per liter:**

Garden soil .....	500.0g
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**Preparation of Soil Extract:** Add 500.0g of garden soil to 1.0L of tap water. Autoclave for 1 hr at 15 psi pressure–121°C. Filter through Whatman #2 filter paper.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Streptomyces rectus*.

**Soil Extract Glycerol Medium****Composition** per liter:

Glycerol .....	20.0g
Peptone.....	5.0g
Beef extract.....	3.0g
Soil extract.....	150.0mL

pH 7.0 ± 0.2 at 25°C

**Soil Extract:****Composition** per liter:

Soil.....	400.0g
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**Preparation of Soil Extract:** Allow garden soil to air dry. Add 400.0g of the air dried garden soil to 960.0mL of tap water. Autoclave for 60 min at 15 psi pressure–121°C. Allow to cool to 25°C. Let stand until settling ceases. Decant the liquid through Whatman filter paper. Autoclave for 30 min at 15 psi pressure–121°C. Cool to room temperature. Let stand until settling ceases.

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Mycobacterium terrae*.

**Soil Extract Medium****Composition** per liter:

Agar .....	15.0g
Pancreatic digest of gelatin.....	5.0g
Beef extract.....	3.0g
Soil extract.....	250.0mL

pH 6.8 ± 0.2 at 25°C

**Soil Extract:****Composition** per liter:

Garden soil.....	500.0g
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**Preparation of Soil Extract:** Add 500.0g of garden soil to 1.0L of tap water. Autoclave for 1 hr at 15 psi pressure–121°C. Filter through Whatman #2 filter paper.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Streptomyces rectus*.

**Soil Extract Medium****Composition** per liter:

Soil.....	400.0g
Agar .....	15.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Sieve air-dried garden soil with a high content of organic matter. To 400.0g of soil add 1.0L of distilled/deionized water. Autoclave for 60 min at 15 psi pressure–121°C. Cool to room temperature. Allow solids to sediment for a few hours. Carefully decant the supernatant solution. Centrifuge the supernatant solution at 10,000 × g for 15 min. Decant supernatant solution. Add agar (15.0g per liter of supernatant). Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Arthrobacter* species, *Aureobacterium flavescens*, *Aureobacterium terregens*, and *Bacillus thiaminolyticus*.

**Soil Extract Peptone Beef Extract Medium****Composition** per liter:

Agar .....	15.0g
Peptone.....	5.0g
Beef extract.....	3.0g
Soil extract.....	1.0L

pH 7.0 ± 0.2 at 25°C

**Soil Extract:****Composition** per liter:

Garden soil .....	400.0g
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**Preparation of Soil Extract:** Add garden soil to 1.0L of tap water. Autoclave for 1 hr at 15 psi pressure–121°C. Filter through cheesecloth and Whatman #2 filter paper. Autoclave filtrate again for 20 min at 15 psi pressure–121°C. Filter through Whatman #2 filter paper.

**Preparation of Medium:** Add agar, peptone, and beef extract to 1.0L of soil extract. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Oerskovia tur-bata* and *Oerskovia xanthineolytica*.

**Soil Extract Potato Extract Medium****Composition** per 510.0mL:

Malt extract .....	10.0g
Yeast extract .....	4.0g
Potato extract.....	250.0mL
Soil extract.....	250.0mL

pH 7.0 ± 0.2 at 25°C

**Soil Extract:****Composition** per liter:

Garden soil .....	400.0g
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**Preparation of Soil Extract:** Add garden soil to 1.0L of tap water. Autoclave for 45 min at 15 psi pressure–121°C. Filter through cheesecloth.

**Potato Extract:****Composition** per liter:

Potatoes .....	400.0g
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**Preparation of Potato Extract:** Peel and dice potatoes. Add 500.0mL of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 15 min. Filter through cheesecloth. Bring volume to 1.0L with distilled/deionized water.

**Preparation of Medium:** Combine components. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Saccharopolyspora rectivirgula*.

**Soil Extract Salts Medium****Composition** per liter:

Soil extract stock .....	625.0mL
Solution 1 .....	125.0mL
Solution 2 .....	125.0mL
Solution 3 .....	125.0mL

**Soil Extract Stock:****Composition** per liter:

Soil, air dried and sieved.....	333.3g
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**Preparation of Soil Extract Stock:** Add soil to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.0 with 1N NaOH or HCl. Autoclave for 30 min at 15 psi pressure–121°C. Allow soil to settle

out. Carefully pour off supernatant. Filter through two layers of cheesecloth.

#### **Solution 1:**

**Composition** per liter:

K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g

**Preparation of Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Solution 2:**

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.0g

**Preparation of Solution 2:** Add MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Solution 3:**

**Composition** per liter:

KNO<sub>3</sub> ..... 10.0g

**Preparation of Solution 3:** Add KNO<sub>3</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Chlamydomonas applanata*, *Polytoma uvella*, *Polytoma mirum*, *Polytoma ellipticum*, *Polytoma difficile*, and *Polytoma anomale*.

### **Soil Seawater Medium for Algae**

**Composition** per liter:

HESNW medium solution ..... 750.0mL

Soil extracts salts medium solution ..... 250.0mL

#### **HESNW Medium Solution:**

**Composition** per 1011.0mL:

Natural seawater ..... 1.0L

Enrichment solution ..... 10.0mL

Vitamin solution ..... 1.0mL

**Preparation of HESNW Medium Solution:** Allow natural seawater to age for 2 months. Filter sterilize. Aseptically add 10.0mL of sterile vitamin solution and 1.0mL enrichment solution. Mix thoroughly.

#### **Enrichment Solution:**

**Composition** per liter:

NaNO<sub>3</sub> ..... 4.667g

Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O ..... 3.000g

Sodium glycerophosphate ..... 0.667g

EDTA·2H<sub>2</sub>O ..... 0.553g

H<sub>3</sub>BO<sub>3</sub> ..... 0.380g

Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O ..... 0.234g

MnSO<sub>4</sub>·4H<sub>2</sub>O ..... 0.054g

FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 0.016g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 7.3mg

CoSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.6mg

**Preparation of Enrichment Solution:** Add 3.000g of Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O to distilled/deionized water. Mix thoroughly. Neutralize Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O with 1N HCl. Add 500.0mL of distilled/deionized water. Mix thoroughly. Add remaining components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Filter sterilize.

#### **Vitamin Solution:**

**Composition** per liter:

Thiamine ..... 0.1g

Vitamin B<sub>12</sub> ..... 2.0mg

Biotin ..... 1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### **Soil Extracts Salts Medium Solution:**

**Composition** per liter:

Soil extract stock ..... 625.0mL

Solution 1 ..... 125.0mL

Solution 2 ..... 125.0mL

Solution 3 ..... 125.0mL

#### **Soil Extract Stock:**

**Composition** per liter:

Soil, air dried and sieved ..... 333.3g

**Preparation of Soil Extract Stock:** Air dry soil. Sieve through fine-mesh screen. Add soil to distilled/deionized water and bring volume to 1.0L. Adjust pH to 8.0 with 1N NaOH or 1N HCl. Autoclave for 30 min at 15 psi pressure–121°C. Allow soil to settle. Decant liquid. Filter through cheesecloth.

#### **Solution 1:**

**Composition** per liter:

K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g

**Preparation of Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Solution 2:**

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.0g

**Preparation of Solution 2:** Add MgSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Solution 3:**

**Composition** per liter:

KNO<sub>3</sub> ..... 10.0g

**Preparation of Solution 3:** Add KNO<sub>3</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Soil Extracts Salts Medium Solution:** Combine 625.0mL of soil extract stock, 125.0mL of solution 1, 125.0mL of solution 2, and 125.0mL of solution 3. Filter sterilize.

**Preparation of Medium:** Aseptically combine 750.0mL of sterile HESNW medium solution and 250.0mL of sterile soil extracts salts medium solution. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Amphora roettgeri*.

### **Sorogena Medium**

**Composition** per 2000.0mL:

HI Agar ..... 1.0L

HI/LV Broth ..... 1.0L

#### **HI Agar:**

**Composition** per liter:

Agar ..... 15.0g

Hay infusion broth ..... 1.0L

pH 6.5 ± 0.2 at 25°C

#### **Hay Infusion Broth:**

**Composition** per liter:

Hay ..... 2.5g

**Preparation of Hay Infusion Broth:** Add hay to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Boil for 30 min. Filter through Whatman #1 filter paper. Bring volume to 1.0L with distilled/deionized water.

**Preparation of HI Agar:** Add hay to hay infusion broth and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 with 5% lactic acid or 1N NaOH. Gently heat and bring to boiling. Autoclave for 20 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

#### HI/LY Broth:

**Composition** per liter:

Lactose .....	0.2g
Yeast extract .....	0.1g
Hay infusion broth .....	1.0L
pH 6.0 ± 0.2 at 25°C	

#### Hay Infusion Broth:

**Composition** per liter:

Hay .....	2.5g
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**Preparation of Hay Infusion Broth:** Add lactose and yeast extract to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Boil for 30 min. Filter through Whatman #1 filter paper. Bring volume to 1.0L with distilled/deionized water.

**Preparation of HI/LY Broth:** Add components to hay infusion broth and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 with 5% lactic acid. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Aseptically add 15.0mL of HI/LY broth as an overlay over the surface of the HI agar plates.

**Use:** For the cultivation of *Sorogena stoianovitchae*.

#### SOT Medium

**Composition** per liter:

NaHCO <sub>3</sub> .....	16.8g
NaNO <sub>3</sub> .....	2.5g
K <sub>2</sub> SO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Disodium EDTA·2H <sub>2</sub> O .....	0.08g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.04g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Trace metals mix A5 .....	1.0mL
Trace metals mix B6, modified .....	1.0mL
pH 9.0 ± 0.2 at 25°C	

#### Trace Metals Mix A5:

**Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of Trace Metals Mix A5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Metals Mix B6, Modified:

**Composition** per liter:

NH <sub>4</sub> NO <sub>3</sub> .....	0.23g
K <sub>2</sub> Cr <sub>2</sub> (SO <sub>4</sub> ) <sub>4</sub> ·24H <sub>2</sub> O .....	0.096g
NiSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.048g
Ti <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	0.04g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	0.018g

**Preparation of Trace Metals Mix B6, Modified:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 9.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Spirulina maxima* and *Spirulina platensis*.

#### Soybean Agar

**Composition** per liter:

White soybeans .....	100.0g
Agar .....	15.0g

**Preparation of Medium:** Add soybeans to 1.0L of distilled/deionized water. Soak overnight. Autoclave for 60 min at 15 psi pressure–121°C. Filter through cheesecloth. Measure volume of filtrate. Add agar to a concentration of 1.5%. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus subtilis* and *Pseudomonas syringae*.

#### SP Agar

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	2.5g
Galactose .....	1.0g
Raffinose .....	1.0g
Sucrose .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
Vitamin solution .....	2.5mL

#### Vitamin Solution:

**Composition** per liter:

Inositol .....	1.0g
Calcium pantothenate .....	0.2g
Choline hydrochloride .....	0.2g
Thiamine .....	0.1g
Nicotinamide .....	0.75g
Pyridoxin .....	0.75g
Riboflavin .....	0.75g
p-Aminobenzoic acid .....	5.0mg
Folic acid .....	1.0mg
Biotin .....	0.05mg
Vitamin B <sub>12</sub> .....	0.05mg
Ethanol .....	1.0L

**Preparation of Vitamin Solution:** Add solid components to 1.0L of ethanol. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

#### SP 2 Agar

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	3.0g
Yeast extract .....	1.0g
Sodium acetate .....	0.02g
Artificial seawater .....	1.0L
pH 7.2 ± 0.2 at 25°C	

#### Artificial Seawater:

**Composition** per liter:

NaCl .....	24.7g
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MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.6g
CaCl <sub>2</sub> .....	1.0g
KCl .....	0.7g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to 1.0L of artificial seawater. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### SP 6 Agar

#### Composition per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	3.0g
Yeast extract .....	1.0g
Artificial seawater .....	1.0L

pH 7.2 ± 0.2 at 25°C

#### Artificial Seawater:

##### Composition per liter:

NaCl .....	24.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.6g
CaCl <sub>2</sub> .....	1.0g
KCl .....	0.7g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to 1.0L of artificial seawater. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### SP5 Broth

#### Composition per liter:

Pancreatic digest of casein .....	9.0g
Yeast extract .....	1.0g
Artificial seawater .....	1.0L

pH 7.2 ± 0.2 at 25°C

#### Artificial Seawater:

##### Composition per liter:

NaCl .....	24.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.6g
CaCl <sub>2</sub> .....	1.0g
KCl .....	0.7g
NaHCO <sub>3</sub> .....	0.2g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add solid components to 1.0L of artificial seawater. Mix thoroughly. Gently heat and

bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### SP Medium

#### Composition per liter:

Agar .....	15.0g
Soluble starch .....	5.0g
Pancreatic digest of casein .....	2.5g
Galactose .....	1.0g
Raffinose .....	1.0g
Sucrose .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Archanigium gephyra*, *Cystobacter fuscus*, *Melittangium lichenicola*, *Myxococcus* species, *Polyangium brachy- sporum*, *Stigmatella aurantiaca*, and *Stigmatella erecta*.

### SP4 Medium

#### Composition per liter:

Base solution .....	615.0mL
Fetal calf serum	
(inactivated at 56°C, 1 hr) .....	170.0mL
Yeast extract (2% solution) .....	100.0mL
CMRL 1066, 10X with glutamine .....	50.0mL
Fresh yeast extract solution .....	35.0mL
Phenol Red (0.1% solution) .....	20.0mL
Penicillin solution .....	10.0mL

pH 7.0–7.4 ± 0.2 at 25°C

#### Base Solution:

##### Composition per 615.0mL:

Pancreatic digest of casein .....	11.2g
Noble agar .....	8.0g
Pancreatic digest of gelatin .....	5.3g
Glucose .....	5.0g
NaCl .....	0.875g
Beef extract .....	0.525g
Yeast extract .....	0.525g
Beef heart, solids from infusion .....	0.35g

**Preparation of Base Solution:** Add components to distilled/deionized water and bring volume to 615.0mL. Mix thoroughly. Adjust pH to 7.5. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### CMRL 1066, 10X with Glutamine:

##### Composition per liter:

NaCl .....	6.8g
NaHCO <sub>3</sub> .....	2.2g
D-Glucose .....	1.0g
KCl .....	0.4g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.26g
CaCl <sub>2</sub> , anhydrous .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O .....	0.14g
L-Glutamine .....	0.1g
Sodium acetate·3H <sub>2</sub> O .....	0.083g
L-Glutamic acid .....	0.075g

L-Arginine-HCl .....	0.07g
L-Lysine-HCl .....	0.07g
L-Leucine .....	0.06g
Glycine .....	0.05g
Ascorbic acid .....	0.05g
L-Proline .....	0.04g
L-Tyrosine .....	0.04g
L-Aspartic acid .....	0.03g
L-Threonine .....	0.03g
L-Alanine .....	0.025g
L-Phenylalanine .....	0.025g
L-Serine .....	0.025g
L-Valine .....	0.025g
L-Cystine .....	0.02g
L-Histidine-HCl·H <sub>2</sub> O .....	0.02g
L-Isoleucine .....	0.02g
Phenol Red .....	0.02g
L-Methionine .....	0.015g
Deoxyadenosine .....	0.01g
Deoxycytidine .....	0.01g
Deoxyguanosine .....	0.01g
Glutathione, reduced .....	0.01g
Thymidine .....	0.01g
Hydroxy-L-proline .....	0.01g
L-Tryptophan .....	0.01g
Nicotinamide adenine dinucleotide .....	7.0mg
Tween 80 .....	5.0mg
Sodium glucuronate·H <sub>2</sub> O .....	4.2mg
Coenzyme A .....	2.5mg
Coccarboxylase .....	1.0mg
Flavin adenine dinucleotide .....	1.0mg
Nicotinamide adenine dinucleotide phosphate .....	1.0mg
Uridine triphosphate .....	1.0mg
Choline chloride .....	0.5mg
Cholesterol .....	0.2mg
5-Methyldeoxycytidine .....	0.1mg
Inositol .....	0.05mg
p-Aminobenzoic acid .....	0.05mg
Niacin .....	0.025mg
Niacinamide .....	0.025mg
Pyridoxine .....	0.025mg
Pyridoxal-HCl .....	0.025mg
Biotin .....	0.01mg
D-Calcium pantothenate .....	0.01mg
Folic acid .....	0.01mg
Riboflavin .....	0.01mg
Thiamine-HCl .....	0.01mg

**Preparation of CMRL 1066, 10X with Glutamine:**  
Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Filter sterilize.

#### **Fresh Yeast Extract Solution:**

##### **Composition per 100.0mL:**

Baker's yeast, live, pressed, starch-free ..... 25.0g

**Preparation of Fresh Yeast Extract Solution:** Add the live Baker's yeast to 100.0mL of distilled/deionized water. Autoclave for 90 min at 15 psi pressure–121°C. Allow to stand. Remove supernatant solution. Adjust pH to 6.6–6.8.

#### **Penicillin Solution:**

##### **Composition per 10.0mL:**

Penicillin G ..... 1,000,000U

**Preparation of Penicillin Solution:** Add penicillin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 615.0mL of cooled sterile base solution, aseptically add 170.0mL of sterile inactivated fetal calf serum, 100.0mL of sterile yeast extract, 50.0mL of sterile CMRL 1066, 10X with glutamine, 35.0mL of sterile fresh yeast extract solution, 20.0mL of Phenol Red solution, and 10.0mL of sterile penicillin solution. Mix thoroughly. Aseptically distribute into sterile tubes. Allow tubes to cool in a slanted position.

**Use:** For the cultivation of tick-derived *Mycoplasma* (*Spiroplasma*). Used for the enhanced recovery of *Mycoplasma pneumoniae*, *Mycoplasma alvi*, and *Mycoplasma hyopneumoniae*.

### ***Sphaerotilus* Agar (DSMZ Medium 51)**

#### **Composition per liter:**

Agar ..... 15.0g  
Beef extract, Lab Lemco ..... 5.0g  
pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool in a sloping position to form slants. Cover solid slants with 2mL sterile tap water. Inoculate into the covering tap water and incubate at 20–25°C.

**Use:** For the cultivation and maintenance of *Sphaerotilus natans*.

### ***Sphaerotilus* CGYA Medium**

#### **Composition per liter:**

Glycerol ..... 10.0g  
Pancreatic digest of casein ..... 5.0g  
Yeast extract ..... 1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Sphaerotilus natans* and *Sphaerotilus* species.

### ***Sphaerotilus* Defined Medium**

#### **Composition per liter:**

Agar ..... 15.0g  
Glycerol ..... 5.0g  
Glutamic acid ..... 0.9g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.03g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.03g  
Phosphate solution ..... 100.0mL  
pH 7.0 ± 0.2 at 25°C

#### **Phosphate Solution:**

##### **Composition per 500.0mL:**

K<sub>2</sub>HPO<sub>4</sub> ..... 5.7g  
KH<sub>2</sub>PO<sub>4</sub> ..... 2.3g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat until dissolved. Autoclave for 15 min at 15 psi pressure–121°C.



**Preparation of Medium:** Add components, except phosphate solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 10 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 100.0mL of sterile phosphate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Sphaerotilus* species.

#### *Sphaerotilus discophorus* Medium

**Composition per liter:**

Agar .....	12.0g
Peptone .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.05g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.05g
Ferric solution .....	100.0mL

pH 7.0 ± 0.2 at 25°C

#### **Ferric Solution:**

**Composition per 100.0mL:**

Ferric ammonium citrate .....	0.5g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Ferric Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ferric solution, to tap water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile ferric solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Sphaerotilus discophorus*.

#### *Sphaerotilus* Isolation Medium

**Composition per liter:**

Agar .....	15.0g
Glycerol .....	10.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Sphaerotilus* species.

#### *Sphaerotilus/Leptothrix* Agar

**Composition per liter:**

Agar .....	20.0g
Peptone .....	1.5g
Yeast extract .....	1.0g
Ferric ammonium citrate .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.05g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.05g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.1. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Leptothrix cholodnii*, *Leptothrix* species, and *Sphaerotilus natans*.

#### *Sphaerotilus* and *Leptothrix* Enrichment Medium

**Composition per liter:**

Glucose .....	1.0g
Peptone .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enrichment and cultivation of *Sphaerotilus* species and *Leptothrix* species.

#### *Sphaerotilus Leptothrix* Medium (DSMZ Medium 803)

**Composition per liter:**

Agar .....	20.0g
Peptone .....	1.5g
Yeast extract .....	1.0g
Ferric ammonium citrate .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.05g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Leptothrix mobilis*.

#### *Sphaerotilus* Medium

**Composition per liter:**

Agar .....	15.0g
Lab-Lemco powder .....	5.0g

pH 7.0 ± 0.2 at 25°C

**Source:** Lab-Lemco powder is available from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Sphaerotilus natans*.

#### *Sphaerotilus natans* Enrichment Medium

**Composition per liter:**

Sodium lactate .....	0.1g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	0.034g
CaCl <sub>2</sub> .....	0.027g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.023g
K <sub>2</sub> HPO <sub>4</sub> .....	0.022g
KH <sub>2</sub> PO <sub>4</sub> .....	8.5mg
NH <sub>4</sub> Cl .....	1.7mg
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.25mg

pH 7.1–7.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enrichment and cultivation of *Sphaerotilus natans*.

#### *Sphaerotilus natans* Isolation Agar

**Composition** per liter:

Agar .....	15.0g
Casein hydrolysate .....	1.5g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Sphaerotilus natans*.

#### *Spirillum gracile* Medium

**Composition** per liter:

Agar .....	15.0g
Peptone .....	5.0g
Yeast extract .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
Tween 80 .....	0.02g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Aquaspirillum gracile*.

#### *Spirillum lipoferum* Medium

**Composition** per liter:

Sodium malate .....	5.0g
Agar .....	3.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
NaCl .....	0.1g
CaCl <sub>2</sub> .....	0.02g
FeCl <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg
Bromothymol Blue solution .....	5.0mL

pH 6.8 ± 0.2 at 25°C

#### Bromothymol Blue Solution:

**Composition** per 10.0mL:

Bromothymol Blue .....	0.5g
Ethanol .....	10.0mL

**Preparation of Bromothymol Blue Solution:** Add Bromothymol Blue to 10.0mL of ethanol. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Spirillum lipoferum*.

#### *Spirillum* Medium

**Composition** per liter:

Calcium lactate .....	10.0g
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Peptone .....	5.0g
Beef extract .....	3.0g
Yeast extract .....	3.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 20 min at 11 psi pressure–116°C. A precipitate will form during autoclaving.

**Use:** For the cultivation of *Spirillum* species.

#### *Spirillum* Medium

**Composition** per liter:

Peptone .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Succinic acid .....	1.0g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Aquaspirillum autotrophicum*, *Aquaspirillum dispar*, *Aquaspirillum peregrinum*, and *Aquaspirillum serpens*.

#### *Spirillum* Nitrogen-Fixing Medium

**Composition** per liter:

Sodium malate .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
K <sub>2</sub> HPO <sub>4</sub> .....	0.1g
NaCl .....	0.1g
Yeast extract .....	0.05g
CaCl <sub>2</sub> .....	0.02g
FeCl <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.0mg

pH 7.2–7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Azospirillum brasilense*, *Azospirillum lipoferum*, and *Herbaspirillum seropedicae*.

#### *Spirillum volutans* Defined Medium

**Composition** per liter:

BES ( <i>N,N</i> -bis[2-hydroxyethyl]-2-aminoethane sulfonic acid) buffer .....	1.07g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Succinic acid .....	1.0g
L-Histidine .....	0.2g
L-Isoleucine .....	0.2g
L-Methionine .....	0.2g
L-Threonine .....	0.2g
NaCl .....	0.085g
L-Cystine .....	0.025g
K <sub>2</sub> HPO <sub>4</sub> .....	0.02g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	3.0mg
DL-Norepinephrine .....	2.0mg

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg
CaCO <sub>3</sub> .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.72mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.245mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.14mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.13mg
H <sub>2</sub> BO <sub>3</sub> .....	0.031mg

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Spirillum volutans*.

### Spirochete Enrichment Medium

**Composition** per liter:

Agar .....	10.0g
Beef extract .....	1.0g
Peptone .....	1.0g
Yeast extract .....	1.0g
Seawater .....	500.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation of spirochetes from muds. A well is cut into the agar plate and filled with mud samples. Spirochetes migrate out of the mud into the agar surrounding the well.

### Spirochete Medium (ATCC Medium 1712)

**Composition** per liter:

Tris(hydroxymethyl)aminomethane buffer .....	7.52g
Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
L-Cysteine-HCl·2H <sub>2</sub> O .....	0.5g
Resazurin .....	1.0mg
Seawater .....	750.0mL
Glucose solution .....	20.0mL

pH 7.2 ± 0.2 at 25°C

### Glucose Solution:

**Composition** per 20.0mL:

Glucose .....	2.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except glucose solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spirochaeta litoralis*.

### Spirochete Thermophile Medium (DSMZ Medium 509)

**Composition** per 1012mL:

Solution A .....	920.0mL
Solution D .....	50.0mL
Solution E .....	20.0mL
Solution F .....	10.0mL

Solution G .....	10.0mL
Solution B .....	1.0mL
Solution C .....	1.0mL

pH 7.0 ± 0.2 at 25°C

### Solution A:

**Composition** per 920.0mL:

NaCl .....	4.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.8g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.03g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to 920.0mL distilled/deionized water. Mix thoroughly. Bring to boiling for a few minutes. Cool to room temperature while gassing with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas. Adjust pH to 6.0. Immediately distribute under N<sub>2</sub> into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### Solution B:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### Solution C:

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution C:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Solution D:

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution D:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

### Solution E:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Solution F:**

**Composition** per 10mL:

Starch ..... 1.0g

**Preparation of Solution F:** Add starch to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution G:**

**Composition** per 10mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Solution A is distributed into anaerobic tubes with rubber stoppers prior to autoclaving. Using aseptic and anaerobic conditions and syringes appropriate volumes of sterile solutions B–G are injected into each tube to yield the specified concentrations.

**Use:** For the cultivation of *Spirochaeta thermophila*.

***Spiroplasma* Agar MID**

**Composition** per 291.2mL:

Schneider's *Drosophila* medium ..... 160.0mL

Solution 1 ..... 80.0mL

Fetal calf serum ..... 50.0mL

Phenol Red (0.5% solution) ..... 1.2mL

pH 7.4 ± 0.2 at 25°C

**Schneider's *Drosophila* Medium:**

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.7g

NaCl ..... 2.1g

Yeast extract ..... 2.0g

Trehalose ..... 2.0g

D-Glucose ..... 2.0g

L-Glutamine ..... 1.8g

L-Lysine-HCl ..... 1.7g

L-Proline ..... 1.7g

KCl ..... 1.6g

Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O ..... 1.3g

L-Glutamic acid ..... 0.8g

L-Methionine ..... 0.8g

CaCl<sub>2</sub>, anhydrous ..... 0.6g

KH<sub>2</sub>PO<sub>4</sub> ..... 0.5g

β-Alanine ..... 0.5g

L-Tyrosine ..... 0.5g

L-Arginine ..... 0.4g

L-Aspartic acid ..... 0.4g

L-Histidine ..... 0.4g

L-Threonine ..... 0.4g

NaHCO<sub>3</sub> ..... 0.4g

Glycine ..... 0.3g

L-Serine ..... 0.3g

L-Valine ..... 0.3g

L-Isoleucine ..... 0.2g

L-Leucine ..... 0.2g

L-Phenylalanine ..... 0.2g

α-Ketoglutaric acid ..... 0.2g

Fumaric acid ..... 0.1g

Malic acid ..... 0.1g

Succinic acid ..... 0.1g

L-Cystine ..... 0.1g

L-Tryptophan ..... 0.1g

L-Cysteine ..... 0.06g

**Preparation of Schneider's *Drosophila* Medium:**

Add components to 1.0L of distilled/deionized water. Mix thoroughly. Filter sterilize.

**Solution 1:**

**Composition** per 80.0mL:

Sorbitol ..... 7.0g

Noble agar ..... 5.0g

Beef heart, solids from infusion ..... 5.0g

Peptone ..... 1.8g

Sucrose ..... 1.0g

Pancreatic digest of casein ..... 1.0g

NaCl ..... 0.5g

D-Fructose ..... 0.1g

D-Glucose ..... 0.1g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Adjust pH to 7.8 with 1N NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Preparation of Medium:** Bring fetal calf serum and Phenol Red solution to 56°C. Rapidly bring Schneider's *Drosophila* medium to 37°C. Rapidly combine the components. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma kunkelii* and *Spiroplasma* species.

***Spiroplasma* Medium**

**Composition** per liter:

Sucrose ..... 80.0g

Beef heart, solids from infusion ..... 34.7g

Peptone ..... 6.9g

NaCl ..... 3.5g

Horse serum, heat inactivated ..... 100.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except horse serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add horse serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma* species.

***Spiroplasma* Medium**

**Composition** per liter:

Sorbitol ..... 70.0g

Pancreatic digest of casein ..... 7.0g

Yeast extract ..... 5.0g

NaCl ..... 5.0g

Beef extract ..... 3.0g

Yeast extract ..... 3.0g

Beef heart, solids from infusion ..... 2.0g

Fructose ..... 1.0g

Glucose ..... 1.0g

Phenol Red ..... 20.0mg

Horse serum ..... 100.0mL

pH 7.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile horse serum. Mix thoroughly.

**Use:** For the cultivation of *Spiroplasma citri*.

***Spiroplasma Medium*  
with 25 mg/L of Phenol Red**

**Composition** per liter:

Sucrose.....	80.0g
Beef heart, solids from infusion.....	34.7g
Peptone.....	6.9g
NaCl.....	3.5g
Phenol Red.....	25.0mg
Horse serum, heat inactivated.....	100.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except horse serum, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add heat-inactivated horse serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Spiroplasma floricola*.

***Sporobacter Medium*  
(DSMZ Medium 711)**

**Composition** per liter:

NH <sub>4</sub> Cl.....	1.0g
NaCl.....	0.6g
Na-acetate·3H <sub>2</sub> O.....	0.5g
Cysteine-HCl·H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
Yeast extract.....	0.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
KCl.....	0.1g
Resazurin.....	0.5mg
NaHCO <sub>3</sub> solution.....	40.0mL
Trimethoxycinnamate solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution SL-10.....	1.5mL

pH 7.1 ± 0.2 at 25°C

**Trace Elements Solution SL-10:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trimethoxycinnamate Solution:**

**Composition** per 10.0mL:

Trans-3,4,5-trimethoxycinnamate.....	1.2g
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**Preparation of Trimethoxycinnamate Solution:**

Add trans-3,4,5-trimethoxycinnamate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Neutralize with NaOH. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, trimethoxycinnamate solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 938.5mL. Mix thoroughly. Adjust pH to 7.1. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 40.0mL NaHCO<sub>3</sub> solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL trimethoxycinnamate solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Sporobacter termitidis*.

***Sporocytophaga Medium***

**Composition** per liter:

NaNO <sub>3</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0g
KCl.....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14g
Yeast extract.....	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	6.0mg
Filter paper strips.....	variable

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except filter paper strips, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes in 5.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add a sterile filter paper strip to each tube so that 1.0–2.0cm of the strip extends above the medium.

**Use:** For the cultivation and maintenance of *Sporocytophaga myxococcoides*.

***Sporohalobacter lortetii* Agar**

**Composition** per liter:

NaCl.....	105.0g
L-Glutamic acid.....	4.0g
Agar.....	20.0g
CaCO <sub>3</sub> .....	5.0g
Soluble starch.....	2.0g
Casamino acids.....	2.0g
Nutrient broth.....	2.0g
Yeast extract.....	2.0g
KCl.....	0.75g
L-Cysteine.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.002g
Resazurin.....	1.0mg
MgCl <sub>2</sub> ·6H <sub>2</sub> O solution.....	40.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution.....	10.0mL

Trace elements solution .....	10.0mL
Vitamin solution.....	10.0mL
pH $6.5 \pm 0.2$ at $25^{\circ}\text{C}$	

**MgCl<sub>2</sub>·6H<sub>2</sub>O Solution:****Composition** per 40.0mL:

MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.01g
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**Preparation of MgCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add 0.01g of MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:****Composition** per 40.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.01g
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**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add 0.01g of CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Vitamin Solution:****Composition** per liter:

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except MgCl<sub>2</sub>·6H<sub>2</sub>O solution and CaCl<sub>2</sub>·2H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 40.0mL of sterile MgCl<sub>2</sub>·6H<sub>2</sub>O solution and 10.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Sporohalobacter lortetii*.

**Sporomusa Medium****Composition** per 1010.0mL:

Betaine·H <sub>2</sub> O.....	6.7g
NaHCO <sub>3</sub> .....	4.0g
NaCl .....	2.25g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
NH <sub>4</sub> Cl .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
NaHSeO <sub>3</sub> .....	26.3μg
Vitamin solution .....	10.0mL
Reducing agent solution .....	10.0mL
Trace elements solution SL-10.....	1.0mL

pH  $7.0 \pm 0.2$  at  $25^{\circ}\text{C}$

**Vitamin Solution:****Composition** per liter:

Pyridoxine·HCl.....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin.....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Reducing Agent Solution:****Composition** per 10.0mL:

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g

**Preparation of Reducing Agent Solution:** Add 10.0mL of distilled/deionized water to a flask. Gently heat and bring to boiling. Continue to boil for 1.0 min while sparging with 100% N<sub>2</sub>. Cool to room temperature. Add L-cysteine·HCl·H<sub>2</sub>O. Mix thoroughly. Adjust pH to 9 with 5N NaOH. Add Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. Autoclave for 10 min at 15 psi pressure–121°C.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except reducing agent solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Using a syringe, aseptically and anaerobically add sterile reducing agent to each tube (10.0mL per liter of medium).

**Use:** For the cultivation and maintenance of *Sporomusa* species.

### *Sporomusa* Medium, Modified

**Composition per liter:**

NaHCO <sub>3</sub> .....	4.0g
NaCl.....	2.25g
Pancreatic digest of casein.....	2.0g
Yeast extract.....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
NH <sub>4</sub> Cl.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0mg
Resazurin.....	1.0mg
NaHSeO <sub>3</sub> .....	26.3μg
Fructose solution.....	50.0mL
Reducing agent solution.....	10.0mL
Vitamin solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL

pH 7.0 ± 0.2 at 25°C

### Fructose Solution:

**Composition per 50.0mL:**

Fructose.....	10.0g
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**Preparation of Fructose Solution:** Add fructose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

### Vitamin Solution:

**Composition per liter:**

Pyridoxine·HCl.....	10.0mg
Calcium DL-pantothenate.....	5.0mg
Lipoic acid.....	5.0mg
Nicotinic acid.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Riboflavin.....	5.0mg
Thiamine·HCl.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### Reducing Agent Solution:

**Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g

**Preparation of Reducing Agent Solution:** Add 10.0mL of distilled/deionized water to a flask. Boil under N<sub>2</sub> gas for 1 min. Cool to room temperature. Add L-cysteine·HCl·H<sub>2</sub>O and dissolve. Adjust to pH 9 with 5*N* NaOH. Add washed Na<sub>2</sub>S·9H<sub>2</sub>O and dissolve. Mix thoroughly. Autoclave for 10 min at 15 psi pressure–121°C.

### Trace Elements Solution SL-10:

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> and reducing agent solution, to distilled/deionized water and bring volume to 940.0mL. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add solid NaHCO<sub>3</sub> and bring pH to 7.0 by gassing. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Prior to inoculation of cultures, aseptically and anaerobically add 0.1mL of sterile reducing agent solution and 0.5mL of sterile fructose solution to each tube containing 9.4mL of sterile basal medium.

**Use:** For the cultivation and maintenance of *Sporomusa acidovorans*.

### *Sporomusa silvacetica* Medium (DSMZ Medium 777)

**Composition per liter:**

NaCl.....	2.25g
Yeast extract.....	1.0g
Casitone.....	1.0g
NH <sub>4</sub> Cl.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.25g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.002g
Resazurin.....	1.0mg
NaHSeO <sub>3</sub> .....	15.1μg
Cysteine solution.....	10.0mL
NaHCO <sub>3</sub> solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Fructose solution.....	10.0mL
Vitamin solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL

pH 6.6 ± 0.2 at 25°C

### Cysteine Solution:

**Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O.....	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Fructose Solution:****Composition per 10.0mL:**

Fructose.....	5.0g
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**Preparation of Fructose Solution:** Add fructose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, fructose solution, cysteine solution, vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust pH to 6.5–6.7. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL fructose solution, 10.0mL cysteine solution, 10.0mL vitamin solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Sporomusa silvacetica*, *Thermicanus aegyptius*, *Moorella thermoacetica*=*Clostridium thermacetium*, and *Moorella mulderi*.

**Sporosarcina halophila Agar****Composition per liter:**

NaCl .....	30.0g
Agar .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.0g
Peptone .....	5.0g
NaCl .....	5.0g
Yeast extract .....	2.0g
Beef extract .....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Halomonas elongata*, *Halomonas halmophila*, *Listonella anguillara*, *Salinicoccus roseus*, *Sporosarcina halophila*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio ordalii*, and *Vibrio vulnificus*.

**Sporosarcina ureae Medium****Composition per liter:**

L-Asparagine-H <sub>2</sub> O or L-glutamine.....	30.0g
KCl .....	3.4g
NaCl .....	2.92g
K <sub>2</sub> HPO <sub>4</sub> .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.05g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.25mg
Biotin solution.....	10.0mL
L-Cysteine solution.....	10.0mL
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> solution .....	10.0mL

pH 8.7 ± 0.2 at 25°C

**Biotin Solution:****Composition per 10.0mL:**

D-Biotin .....	1.0mg
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**Preparation of Biotin Solution:** Add biotin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**L-Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine.....	0.04g
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**Preparation of L-Cysteine Solution:** Add L-cysteine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Solution:****Composition per 10.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
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**Preparation of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Solution:** Add (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except biotin solution, L-cysteine solution, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 8.7 with 1N NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile biotin solution, L-cysteine solution, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes.

**Use:** For the cultivation of *Sporosarcina ureae*.



***Sporosarcina ureae* Medium****Composition per liter:**

Agar .....	30.0g
Glucose .....	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	4.0g
Malt extract .....	3.0g
Peptone .....	3.0g
Yeast extract .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.8g
CaCl <sub>2</sub> .....	0.1g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
ZnSO <sub>4</sub> .....	0.01g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0mg

**Preparation of Medium:** Add components to 1.0L of distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and induction of sporulation of *Sporosarcina ureae*.

**SRB-Psychrophile Medium  
(DSMZ Medium 861)****Composition per 1158mL:**

NaCl .....	20.0g
Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
KBr .....	0.09g
KCl .....	0.5g
Resazurin .....	0.5mg
NH <sub>4</sub> Cl solution .....	49.5mL
KH <sub>2</sub> PO <sub>4</sub> solution .....	49.5mL
NaHCO <sub>3</sub> solution .....	29.7mL
Vitamin solution .....	9.9mL
Substrate solution .....	9.9mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	9.9mL
Dithionite solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL
Selenite-tungstate solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

**Dithionite Solution:****Composition per 10mL:**

Na-dithionite .....	0.25g
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**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg

Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Selenite-Tungstate Solution:****Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**NaHCO<sub>3</sub> Solution:****Composition per 100.0mL:**

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**NH<sub>4</sub>Cl Solution:****Composition per 100.0mL:**

NH <sub>4</sub> Cl .....	0.5g
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**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**KH<sub>2</sub>PO<sub>4</sub> Solution:****Composition per 100.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Substrate Solution:****Composition per 10.0mL:**

Na-acetate .....	1.5g
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**Preparation of Substrate Solution:** Add Na-acetate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except

NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, substrate solution, selenite-tungstate solution, NH<sub>4</sub>Cl solution, KH<sub>2</sub>PO<sub>4</sub> solution, vitamin solution, dithionite solution, and trace elements solution SL-10. Distribute 30.0mL aliquots into 50mL serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically for each 30.0mL medium add 0.9mL NaHCO<sub>3</sub> solution, 0.3mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 0.3mL substrate solution, 0.03mL selenite-tungstate solution, 1.5mL NH<sub>4</sub>Cl solution, 1.5mL KH<sub>2</sub>PO<sub>4</sub> solution, 0.3mL vitamin solution, 0.03mL dithionite solution, and 0.03mL trace elements solution SL-10. Final pH is 7.2.

**Use:** For the cultivation of *Desulfofrigus oceanense*.

### SRB-Psychrophile Medium (DSMZ Medium 861)

#### Composition per 1158mL:

NaCl .....	20.0g
Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KCl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
KBr .....	0.09g
Resazurin .....	0.5mg
NH <sub>4</sub> Cl solution .....	49.5mL
KH <sub>2</sub> PO <sub>4</sub> solution .....	49.5mL
NaHCO <sub>3</sub> solution .....	29.7mL
Vitamin solution .....	9.9mL
Substrate solution .....	9.9mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	9.9mL
Dithionite solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL
Selenite-tungstate solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Dithionite Solution:

##### Composition per 10mL:

Na-dithionite .....	0.25g
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**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Selenite-Tungstate Solution:

##### Composition per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### NH<sub>4</sub>Cl Solution:

##### Composition per 100.0mL:

NH <sub>4</sub> Cl .....	0.5g
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**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### KH<sub>2</sub>PO<sub>4</sub> Solution:

##### Composition per 100.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Substrate Solution:

##### Composition per 10.0mL:

Na-lactate .....	2.5g
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**Preparation of Substrate Solution:** Add Na-lactate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, substrate solution, selenite-tungstate solution, NH<sub>4</sub>Cl solution, KH<sub>2</sub>PO<sub>4</sub> solution, vitamin solution, dithionite solution, and trace elements solution SL-10. Distribute 30.0mL aliquots into 50mL serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically for each 30.0mL medium add 0.9mL NaHCO<sub>3</sub> solution, 0.3mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 0.3mL substrate solution, 0.03mL selenite-tungstate solution, 1.5mL

NH<sub>4</sub>Cl solution, 1.5mL KH<sub>2</sub>PO<sub>4</sub> solution, 0.3mL vitamin solution, 0.03mL dithionite solution, and 0.03mL trace elements solution SL-10. Final pH is 7.2.

**Use:** For the cultivation of *Desulfotalea arctica* and *Desulfofrigus fragile*.

### SRB-Psychrophile Medium (DSMZ Medium 861)

**Composition** per 1158mL:

NaCl	10.0g
Na <sub>2</sub> SO <sub>4</sub>	4.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.0g
KCl	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
KBr	0.09g
Resazurin	0.5mg
NH <sub>4</sub> Cl solution	49.5mL
KH <sub>2</sub> PO <sub>4</sub> solution	49.5mL
NaHCO <sub>3</sub> solution	29.7mL
Vitamin solution	9.9mL
Substrate solution	9.9mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	9.9mL
Dithionite solution	1.0mL
Trace elements solution SL-10	1.0mL
Selenite-tungstate solution	1.0mL

pH 7.2 ± 0.2 at 25°C

### Dithionite Solution:

**Composition** per 10mL:

Na-dithionite	0.25g
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**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

### Selenite-Tungstate Solution:

**Composition** per liter:

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume

to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### NaHCO<sub>3</sub> Solution:

**Composition** per 100.0mL:

NaHCO <sub>3</sub>	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

### NH<sub>4</sub>Cl Solution:

**Composition** per 100.0mL:

NH <sub>4</sub> Cl	0.5g
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**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### KH<sub>2</sub>PO<sub>4</sub> Solution:

**Composition** per 100.0mL:

KH <sub>2</sub> PO <sub>4</sub>	0.4g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Substrate Solution:

**Composition** per 10.0mL:

Na-lactate	2.5g
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**Preparation of Substrate Solution:** Add Na-lactate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, substrate solution, selenite-tungstate solution, NH<sub>4</sub>Cl solution, KH<sub>2</sub>PO<sub>4</sub> solution, vitamin solution, dithionite solution, and trace elements solution SL-10. Distribute 30.0mL aliquots into 50mL serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically for each 30.0mL medium add 0.9mL NaHCO<sub>3</sub> solution, 0.3mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 0.3mL substrate solution, 0.03mL selenite-tungstate solution, 1.5mL NH<sub>4</sub>Cl solution, 1.5mL KH<sub>2</sub>PO<sub>4</sub> solution, 0.3mL vitamin solution, 0.03mL dithionite solution, and 0.03mL trace elements solution SL-10. Final pH is 7.2.

**Use:** For the cultivation of *Desulfotalea psychrophila*.

### SRB-Psychrophile Medium (DSMZ Medium 861)

#### Composition per 1158mL:

NaCl	20.0g
Na <sub>2</sub> SO <sub>4</sub>	4.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
KBr	0.09g
KCl	0.5g
Resazurin	0.5mg
NH <sub>4</sub> Cl solution	49.5mL
KH <sub>2</sub> PO <sub>4</sub> solution	49.5mL
NaHCO <sub>3</sub> solution	29.7mL
Vitamin solution	9.9mL
Substrate solution	9.9mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	9.9mL
Dithionite solution	1.0mL
Trace elements solution SL-10	1.0mL
Selenite-tungstate solution	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Dithionite Solution:

##### Composition per 10mL:

Na-dithionite	0.25g
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**Preparation of Dithionite Solution:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Selenite-Tungstate Solution:

##### Composition per liter:

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg

ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub>	10.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### NH<sub>4</sub>Cl Solution:

##### Composition per 100.0mL:

NH <sub>4</sub> Cl	0.5g
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**Preparation of NH<sub>4</sub>Cl Solution:** Add NH<sub>4</sub>Cl to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### KH<sub>2</sub>PO<sub>4</sub> Solution:

##### Composition per 100.0mL:

KH <sub>2</sub> PO <sub>4</sub>	0.4g
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**Preparation of KH<sub>2</sub>PO<sub>4</sub> Solution:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Substrate Solution:

##### Composition per 10.0mL:

Na-propionate	1.5g
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**Preparation of Substrate Solution:** Add Na-propionate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, substrate solution, selenite-tungstate solution, NH<sub>4</sub>Cl solution, KH<sub>2</sub>PO<sub>4</sub> solution, vitamin solution, dithionite solution, and trace elements solution SL-10. Distribute 30.0mL aliquots into 50mL serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically for each 30.0mL medium add 0.9mL NaHCO<sub>3</sub> solution, 0.3mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 0.3mL substrate solution, 0.03mL selenite-tungstate solution, 1.5mL NH<sub>4</sub>Cl solution, 1.5mL KH<sub>2</sub>PO<sub>4</sub> solution, 0.3mL vitamin solution, 0.03mL dithionite solution, and 0.03mL trace elements solution SL-10. Final pH is 7.2.

**Use:** For the cultivation of *Desulfobaba gelida*.

### SSL Agar

#### Composition per liter:

Agar	2.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.0g
Gelatin	1.0g
KNO <sub>3</sub>	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.0g
NaCl	1.0g
Pancreatic digest of casein	1.0g
Yeast extract	1.0g

Sodium glycerophosphate .....	0.1g
Cyanocobalamin .....	1.0µg
Trace elements solution .....	1.0mL
pH 7.5 ± 0.2 at 25°C	

**Trace Elements Solution:****Composition per liter:**

Disodium EDTA .....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
KBr .....	0.02g
KI .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
LiCl .....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### StA (Schmittenner's Agar)

**Composition per liter:**

Agar .....	20.0g
Sucrose .....	2.5g
Asparagine .....	0.27g
KH <sub>2</sub> PO <sub>4</sub> .....	0.15g
K <sub>2</sub> HPO <sub>4</sub> .....	0.15g
MgSO <sub>4</sub> .....	0.1g
Sitosterol .....	0.01g

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add agar. Swirl. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pythium aph- anidermatum*, *Pythium graminicola*, *Pythium myriotylum*, *Pythium sylvaticum*, and *Pythium ultimum*.

### Staley's Maintenance Agar

**Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
Yeast extract .....	0.5g
Hutner's basal salts solution .....	20.0mL
Vitamin solution .....	10.0mL

**Hutner's Basal Salts Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitritotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>7</sub> ·O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44" .....	50.0mL

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Vitamin Solution:****Composition per liter:**

Nicotinamide .....	9.0mg
Calcium DL-pantothenate .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Hutner's basal salts solution and vitamin solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0 mL of sterile Hutner's basal salts solution and 10.0mL of sterile vitamin solution. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Gemmata obscuriglobus*.

### Stan 4 Agar

**Composition per liter:**

Solution B .....	650.0mL
Solution A .....	350.0mL

**Solution A:****Composition per 350.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
KNO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Trace elements solution .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 350.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution:****Composition per liter:**

EDTA .....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> .....	0.02g
KBr .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g

H <sub>3</sub> BO <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
BaCl <sub>2</sub> .....	5.0mg
LiCl .....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution B:

**Composition per 650.0mL:**

Agar .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 650.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine 350.0mL of cooled, sterile solution A and 650.0mL of cooled, sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of myxobacteria.

#### Stan 5 Agar

**Composition per liter:**

Solution B .....	650.0mL
Solution A .....	350.0mL

#### Solution A:

**Composition per 350.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Trace elements solution .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 350.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### Trace Elements Solution:

**Composition per liter:**

EDTA .....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> .....	0.02g
KBr .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
BaCl <sub>2</sub> .....	5.0mg
LiCl .....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution B:

**Composition per 650.0mL:**

Agar .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 650.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically combine 350.0mL of cooled, sterile solution A and 650.0mL of cooled, sterile so-

lution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of myxobacteria.

#### Stan 6 Agar

**Composition per liter:**

Agar .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
FeCl <sub>3</sub> .....	0.2g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Yeast extract .....	0.02g
Trace elements solution .....	1.0mL

#### Trace Elements Solution:

**Composition per liter:**

Disodium EDTA .....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
KBr .....	0.02g
KI .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
LiCl .....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

#### Standard Methods Agar (Tryptone Glucose Yeast Agar) (Plate Count Agar)

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	2.5g
Glucose .....	1.0g

pH 7.0 ± 0.1 at 25°C

**Source:** Available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and enumeration by microbial plate counts of microorganisms isolated from water and other specimens.

#### Stanier's Basal Medium with Pyridoxine and Yeast Extract

**Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	2.78g
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Na <sub>2</sub> HPO <sub>4</sub> .....	2.78g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Yeast extract.....	0.2g
Hutner's mineral base .....	20.0mL
Pyridoxine solution .....	10.0mL

pH 6.8 ± 0.2 at 25°C

**Pyridoxine Solution:****Composition per 10.0mL:**

Pyridoxine .....	2.0g
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**Preparation of Pyridoxine Solution:** Add pyridoxine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Hutner's Mineral Base:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitritotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

**Preparation of Hutner's Mineral Base:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L.

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except pyridoxine solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 6.8 with 1N KOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile pyridoxine solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

### Stanier's Basal Medium with Trichlorophenoxyacetate

**Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	2.78g
Na <sub>2</sub> HPO <sub>4</sub> .....	2.78g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
2,4,5-Trichlorophenoxyacetate .....	1.0g
Hutner's mineral base .....	20.0mL

**Hutner's Mineral Base:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitritotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> .....	9.25mg
"Metals 44" .....	50.0mL

**Preparation of Hutner's Mineral Base:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Readjust pH to 7.2 with H<sub>2</sub>SO<sub>4</sub> or KOH. Add distilled/deionized water to 1.0L. Store at 5°C.

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
EDTA .....	0.25g
MnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.018g

**Preparation of "Metals 44":** Add a few drops of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water to inhibit precipitate formation. Add components to acidified distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Pseudomonas cepacia*.

**Starch Agar****Composition per liter:**

Starch, soluble .....	20.0g
Agar .....	10.0g
NaNO <sub>3</sub> .....	2.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
NaCl .....	0.1g
FeCl <sub>3</sub> .....	1mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

**Starch Hydrolysis Agar****Composition per liter:**

Beef heart, infusion from .....	500.0g
Soluble starch .....	20.0g
Agar .....	15.0g
Tryptose .....	10.0g
NaCl .....	5.0g

pH 7.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and differentiation of a variety of microorganisms based on amylase production. After incubation, starch hydrolysis is determined by the addition of Gram's or Lugol's iodine solution. Organisms that produce amylase appear as colonies surrounded by a clear zone.

**Starkey's Medium C, Modified****Composition per liter:**

Sodium lactate	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.0g
Na <sub>2</sub> SO <sub>4</sub>	1.0g
NH <sub>4</sub> Cl	1.0g
Yeast extract	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
Ferrous ammonium sulfate solution	50.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution	10.0mL
pH 7.5 ± 0.2 at 25°C	

**Ferrous Ammonium Sulfate Solution:****Composition per 100.0mL:**

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	1.0g
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**Preparation of Ferrous Ammonium Sulfate Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**L-Cysteine-HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O	0.75g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ferrous ammonium sulfate solution and L-cysteine-HCl·H<sub>2</sub>O solution, to tap water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 50.0mL of sterile ferrous ammonium sulfate solution and 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 7.5 with filter-sterilized 2N NaOH. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Desulfotomaculum* species and *Desulfovibrio* species.

**Starkey's Medium C, Modified with Salt****Composition per liter:**

NaCl	25.0g
Sodium lactate	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.0g
Na <sub>2</sub> SO <sub>4</sub>	1.0g
NH <sub>4</sub> Cl	1.0g
Yeast extract	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
Ferrous ammonium sulfate solution	50.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution	10.0mL
pH 7.5 ± 0.2 at 25°C	

**Ferrous Ammonium Sulfate Solution:****Composition per 100.0mL:**

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	1.0g
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**Preparation of Ferrous Ammonium Sulfate Solution:** Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**L-Cysteine-HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O	0.75g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except ferrous ammonium sulfate solution and L-cysteine-HCl·H<sub>2</sub>O solution, to tap water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 50.0mL of sterile ferrous ammonium sulfate solution and 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 7.5 with filter-sterilized 2N NaOH. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of halophilic *Desulfovibrio* species.

**Stetteria Medium (DSMZ Medium 795)****Composition per liter:**

Sulfur, powdered	10.0g
Peptone	2.0g
Yeast extract	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
NaHCO <sub>3</sub>	0.16g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	3.0mg
Resazurin	0.75mg
Synthetic seawater, concentrated	500.0mL
Trace elements solution	15.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Selenite-tungstate solution	1.5mL
pH 7.2 ± 0.2 at 25°C	

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Selenite-Tungstate Solution:****Composition per liter:**

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Synthetic Seawater, Concentrated:**

NaCl	55.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	14.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.5g
KCl	1.3g
NaBr	0.2g
H <sub>3</sub> BO <sub>3</sub>	0.06g
SrCl <sub>2</sub> ·6H <sub>2</sub> O	0.03g
Na <sub>3</sub> -citrate	20.0mg
KI	0.1mg

**Preparation of Synthetic Seawater, Concentrated:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitilotriacetic acid	1.5g



NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except synthetic seawater and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to 490.0mL distilled/deionized water. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 500.0mL filter sterilized concentrated seawater. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture for 20 min. Aseptically add 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Adjust pH to 6.0 with H<sub>2</sub>SO<sub>4</sub>. Mix thoroughly. Aseptically and anaerobically distribute 20mL aliquots into sterile 100mL serum bottles. Pressurize bottles to 2 bar gas overpressure with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Heat at 100°C for 1.5 hr. Before use, check that the medium pH is 6.0.

**Use:** For the cultivation of *Stetteria hydrogenophila* and *Staphylothermus hellenicus*.

### STL Broth

#### Composition per liter:

Casamino acids .....	1.0g
Glucose .....	1.0g
Sodium glutamate .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KNO <sub>3</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Sodium glycerophosphate .....	0.1g
Thiamine .....	1.0mg
Vitamin B <sub>12</sub> .....	1.0μg
Trace elements solution .....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

Sodium EDTA .....	8.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
KBr .....	0.02g
KI .....	0.02g
ZnCl <sub>2</sub> .....	0.02g
CuSO <sub>4</sub> .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
LiCl .....	5.0mg
SnCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Cytophaga* species, *Herpetosiphon* species, *Saprospira* species, and *Flexithrix* species.

### Stokes Agar

#### Composition per liter:

Agar .....	12.5g
Glucose .....	1.0g
Peptone .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> .....	0.05g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Sphaerotilus natans*.

### Straw DYAA

#### Composition per liter:

Agar .....	20.0g
Glucose .....	10.0g
Yeast extract .....	1.0g
Asparagine .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
FeCl <sub>3</sub> solution .....	0.5mL
Straw .....	variable

#### FeCl<sub>3</sub> Solution:

##### Composition per 10.0mL:

FeCl <sub>3</sub> .....	1.0g
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except straw, to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Autoclave straw for 15 min at 15 psi pressure–121°C. Aseptically add straw to the solidified agar.

**Use:** For the cultivation of *Cochliobolus sativus*.

### Straw Malt Agar

#### Composition per liter:

Agar .....	15.0g
Malt extract .....	10.0g
Straw .....	variable

**Preparation of Medium:** Add components, except straw, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Autoclave straw for 15 min at 15 psi pressure–121°C. Aseptically add some straw to the solidified agar.

**Use:** For the cultivation of *Cladosporium vignae*, *Cochliobolus sativus*, and *Cochliobolus victoriae*.

**Stuart *Leptospira* Broth, Modified****Composition per liter:**

NaCl .....	1.93g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.66g
NH <sub>4</sub> Cl .....	0.34g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
L-Asparagine .....	0.13g
KH <sub>2</sub> PO <sub>4</sub> .....	0.08g
Glycerol .....	5.0mL
Rabbit serum, inactivated at 56°C, 30 min .....	100.0mL
pH 7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add each component, except rabbit serum, to distilled/deionized water in separate flasks and bring each volume to 100.0mL. Mix thoroughly. Combine the seven solutions, except the rabbit serum. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile rabbit serum. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Leptospira* species.

**Stuart Medium Base****Composition per 1100.0mL:**

NaCl .....	1.8g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.67g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.41g
NH <sub>4</sub> Cl .....	0.27g
Asparagine .....	0.13g
KH <sub>2</sub> PO <sub>4</sub> .....	0.09g
Phenol Red .....	0.01g
Glycerol .....	5.0mL
<i>Leptospira</i> enrichment .....	100.0mL
pH 7.6 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems. *Leptospira* enrichment contains rabbit serum and hemoglobin and is available from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except glycerol and *Leptospira* enrichment, to distilled/deionized water and bring volume to 995.0mL. Mix thoroughly. Add glycerol. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add *Leptospira* enrichment. Mix thoroughly. Aseptically distribute into sterile screw-capped tubes in 10.0mL volumes.

**Use:** For the cultivation of *Leptospira* species.

**Styrene Mineral Salts Agar****Composition per liter:**

Agar .....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.55g
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O .....	0.85g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
EDTA .....	10.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0mg
ZnSO <sub>4</sub> .....	2.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.2mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or

flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes. Place plates in a desiccator. Add to the desiccator an open bottle containing 10.0mL of dibutyl phthalate and 200.0μl of styrene.

**Use:** For the cultivation of styrene-utilizing microorganisms.

**Sucrose Teepol Tellurite Agar (STT Agar)****Composition per liter:**

Agar .....	20.0g
Beef extract .....	1.0g
Peptone .....	1.0g
Sucrose .....	1.0g
NaCl .....	0.5g
Bromothymol Blue (0.2% solution) .....	2.5mL
Tellurite solution .....	2.5mL
Sodium lauryl sulfate (Teepol, 0.1% solution) .....	0.2mL
pH 8.0 ± 0.2 at 25°C	

**Tellurite Solution:****Composition per 100.0mL:**

K <sub>2</sub> TeO <sub>3</sub> .....	0.05g
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**Preparation of Tellurite Solution:** Add the K<sub>2</sub>TeO<sub>3</sub> to distilled/deionized water and bring the volume to 100.0mL. Mix thoroughly. Filter sterilize. Use freshly prepared solution.

**Caution:** Potassium tellurite is toxic.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Pour into sterile Petri dishes.

**Use:** For the selective isolation, cultivation, and differentiation of *Vibrio* species based on their ability to ferment sucrose. *Vibrio cholerae* appears as flat yellow colonies. *Vibrio parahaemolyticus* appears as elevated green-yellow mucoid colonies.

**Sulfate API Broth****Composition per liter:**

NaCl .....	10.0g
Sodium lactate .....	5.2g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Ascorbic acid .....	0.1g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.01g
pH 7.5 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add the components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly until dissolved. Distribute into tubes in 9.0mL volumes. Autoclave for 10 min at 15 psi pressure–121°C.

**Use:** For the detection, differentiation, and estimation of sulfate-reducing bacteria.

**Sulfate-Reducing Bacteria Enrichment Medium****Composition per 1018.0mL:**

Solution 1 .....	970.0mL
Solution 4 .....	30.0mL
Solution 6A, 6B, 6C, 6D, or 6E .....	10.0mL
Solution 5 .....	3.0mL

Solution 2 .....	1.0mL
Solution 3 .....	1.0mL
Solution 7 .....	1.0mL
Solution 8 .....	1.0mL
Solution 9 .....	1.0mL

pH  $7.2 \pm 0.2$  at 25°C**Solution 1:****Composition** per 970.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
KCl .....	0.3g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Autoclave for 30 min at 15 psi pressure–121°C. Cool to 25°C under 90% N<sub>2</sub> + 10% CO<sub>2</sub>.

**Solution 2:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.12g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.07g
H <sub>3</sub> BO <sub>3</sub> .....	0.06g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.015g
HCl (25% solution) .....	6.5mL

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 3:****Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> .....	3.0mg

**Preparation of Solution 3:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 4:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	8.5g
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**Preparation of Solution 4:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Saturate with 100% CO<sub>2</sub>. Filter sterilize. Aseptically add solution to sterile, gas-tight, screw-capped bottles.

**Solution 5:****Composition** per 100.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	12.0g
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**Preparation of Solution 5:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Add solution to gas-tight, screw-capped bottles. Gas under 100% N<sub>2</sub> for 20 min. Close caps tightly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 6A:****Composition** per 100.0mL:

Sodium acetate·3H <sub>2</sub> O .....	20.0g
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**Preparation of Solution 6A:** Add sodium acetate·3H<sub>2</sub>O to distilled/deionized water and bring volume to

100.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 6B:****Composition** per 100.0mL:

<i>n</i> -Butyric acid .....	8.0g
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**Preparation of Solution 6B:** Add *n*-butyric acid to distilled/deionized water and bring volume to 100.0mL. Adjust pH to 9.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 6C:****Composition** per 100.0mL:

Propionic acid .....	7.0g
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**Preparation of Solution 6C:** Add propionic acid to 100.0mL of distilled/deionized water. Adjust pH to 9.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 6D:****Composition** per 100.0mL:

Benzoic acid .....	5.0g
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**Preparation of Solution 6D:** Add benzoic acid to distilled/deionized water and bring volume to 100.0mL. Adjust pH to 9.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 6E:****Composition** per 100.0mL:

<i>n</i> -Palmitic acid .....	5.0g
NaOH .....	0.78g

**Preparation of Solution 6E:** Add *n*-palmitic acid and NaOH to distilled/deionized water and bring volume to 100.0mL. Heat in a water bath until clear. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 7:****Composition** per 100.0mL:

Thiamine .....	0.01g
<i>p</i> -Aminobenzoic acid .....	5.0mg
Vitamin B <sub>12</sub> .....	5.0mg
Biotin .....	1.0mg

**Preparation of Solution 7:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution 8:****Composition** per liter:

Succinic acid .....	0.6g
Isobutyric acid .....	0.5g
2-Methylbutyric acid .....	0.5g
3-Methylbutyric acid .....	0.5g
Valeric acid .....	0.5g
Caproic acid .....	0.2g

**Preparation of Solution 8:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 9.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution 9:****Composition** per 100.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	3.0g
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**Preparation of Solution 9:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to 100.0mL of O<sub>2</sub>-free distilled/deionized water. Mix thoroughly. Anaerobically filter sterilize.

**Preparation of Medium:** To 970.0mL of cooled, sterile solution 1, aseptically and anaerobically add 1.0mL of sterile solution 2, 1.0mL of sterile solution 3, 30.0mL of sterile

solution 4, and 3.0mL of sterile solution 5. Mix thoroughly. Adjust pH to 7.2 with sterile HCl solution or sterile Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically and anaerobically distribute into sterile screw-capped bottles in 100.0mL volumes. Add 1.0mL of solutions 6A, 6B, 6C, 6D, or 6E to each bottle containing 100.0mL of basal medium. Add 0.1mL of solution 7, 0.1mL of solution 8, and 0.1mL of solution 9 to each bottle containing 100.0mL of basal medium. Mix thoroughly.

**Use:** For the isolation, cultivation, and enrichment of sulfate-reducing bacteria.

### Sulfate-Reducing Bacteria Medium

#### Composition per 1009.0mL:

Solution A .....	850.0mL
Solution C .....	100.0mL
Solution G .....	20.0mL
Solution D .....	10.0mL
Solution E (Wolfe's vitamin solution) .....	10.0mL
Solution H .....	10.0mL
Solution F .....	6.6mL
Solution B (Trace elements solution SL-10).....	1.0mL
Solution I.....	0.4mL

pH 7.6 ± 0.2 at 25°C

#### Solution A:

##### Composition per 920.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction, and a pH of 6.0 is reached. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution B (Trace Elements Solution SL-10):

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.10g
ZnCl <sub>2</sub> .....	0.070g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.036g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.024g
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add the FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Bring volume to approximately 900.0mL with distilled/deionized water. Mix thoroughly. Adjust pH to 6.0 with NaOH. Bring volume to 1.0L with distilled/deionized water. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution C:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thor-

oughly. Filter sterilize. Aseptically gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 20 min.

#### Solution D:

##### Composition per 10.0mL:

Sodium propionate .....	1.5g
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**Preparation of Solution D:** Prepare and dispense solution anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add sodium propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution E (Wolfe's Vitamin Solution):

##### Composition per liter:

Pyridoxine-HCl .....	0.01g
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	0.1mg

#### Preparation of Solution E (Wolfe's Vitamin Solution):

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min.

#### Solution F:

##### Composition per 6.6mL:

AlCl <sub>3</sub> ·6H <sub>2</sub> O (4.9% solution) .....	5.0mL
Na <sub>2</sub> CO <sub>3</sub> (10.6% solution) .....	1.6mL

**Preparation of Solution F:** Combine both solutions. Mix thoroughly. Gas with 100% N<sub>2</sub>. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution G:

##### Composition per 10.0mL:

Rumen fluid, clarified .....	20.0mL
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**Preparation of Solution G:** Gas rumen fluid under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution H:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution H:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Gas under 100% N<sub>2</sub> for 20 min. Cap with a rubber stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Solution I:

##### Composition per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.5g
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**Preparation of Solution I:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically gas under 100% N<sub>2</sub> for 20 min. Prepare solution freshly.

**Preparation of Medium:** To 850.0mL of cooled, sterile solution A, aseptically and anaerobically add in the following order: 1.0mL of sterile solution B, 1000.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, 6.6mL of sterile solution F, 20.0mL of sterile solution G, and 10.0mL of sterile solution H. Mix thoroughly. Immediately prior to inoculation, aseptically and anaerobically add 0.4mL of sterile solution I. Mix thoroughly. Asep-

tically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of a variety of sulfate-reducing bacteria.

### Sulfate-Reducing Bacteria Medium with Lactate

#### Composition per liter:

Solution 1 .....	980.0mL
Solution 2 .....	10.0mL
Solution 3 .....	10.0mL

pH 7.4 ± 0.2 at 25°C

#### Solution 1:

##### Composition per 980.0mL:

Sodium lactate (70% solution) .....	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.0g
Yeast extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

#### Solution 2:

##### Composition per 10.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
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**Preparation of Solution 2:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

#### Solution 3:

##### Composition per 10.0mL:

Ascorbic acid .....	0.1g
Sodium thioglycolate .....	0.1g

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Preparation of Medium:** Aseptically combine 980.0mL of cooled, sterile solution 1, 10.0mL of cooled, sterile solution 2, and 10.0mL of cooled, sterile solution 3. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the enrichment and isolation of sulfate-reducing bacteria.

### Sulfate-Reducing Medium

#### Composition per liter:

Sodium lactate .....	3.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
Peptone .....	2.0g
Na <sub>2</sub> SO <sub>4</sub> .....	1.5g
Beef extract .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> .....	0.1g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O solution .....	10.0mL
Sodium ascorbate solution .....	10.0mL

pH 7.5 ± 0.3 at 25°C

#### Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O Solution:

##### Composition per 100.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	3.92g
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#### Preparation of Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O Solution:

Add Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Use freshly prepared medium.

#### Sodium Ascorbate Solution:

##### Composition per 100.0mL:

Sodium ascorbate .....	0.05g
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**Preparation of Sodium Ascorbate Solution:** Add sodium ascorbate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Use freshly prepared medium.

**Preparation of Medium:** Add components, except Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O solution and sodium ascorbate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Distribute into screw-capped tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Tubes must be filled to capacity after inoculation, so prepare extra medium and sterilize in a screw-capped flask or bottle. Prior to inoculation, aseptically add 0.1mL of freshly prepared sterile Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O solution for each 10.0mL of medium in the tubes. Also aseptically add 0.1mL of freshly prepared sterile sodium ascorbate solution for each 10.0mL of medium in the tubes. Inoculate tubes. Fill tubes to capacity with extra sterile medium. Screw caps tight.

**Use:** For the isolation, cultivation, and enumeration of iron and sulfur bacteria.

### Sulfite Agar

#### Composition per liter:

Agar .....	20.0g
Pancreatic digest of casein .....	10.0g
Na <sub>2</sub> SO <sub>3</sub> .....	1.0g
Iron nails .....	.66

pH 7.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into screw-capped tubes in 15.0mL volumes. Add a clean iron nail to each tube. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C until ready to inoculate.

**Use:** For the detection and cultivation of thermophilic anaerobes that can produce H<sub>2</sub>S from sulfite. Sulfite reduction appears as a blackening of the medium.

### Sulfitobacter pontiacus Medium (DSMZ Medium 733)

#### Composition per liter:

Basal salts solution .....	959.0mL
Biotin solution .....	10.0mL
Na-acetate solution .....	10.0mL
Yeast extract peptone solution .....	10.0mL
Magnesium calcium solution .....	10.0mL
Trace elements solution .....	1.0mL

pH 7.6 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly. Filter sterilize.

#### Basal Salts Solution:

##### Composition per liter:

HEPES .....	8.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
NaCl .....	15.0g

**Preparation of Basal Salts Solution:** Add components to 1.0L of distilled/deionized water. Adjust pH to 7.5–7.8 with NaOH. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

#### Biotin Solution:

##### Composition per 10.0mL:

Biotin .....	0.1g
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**Preparation of Biotin Solution:** Add biotin to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

#### Magnesium Calcium Solution:

##### Composition per 10.0mL:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g

**Preparation of Magnesium Calcium Solution:** Add components to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

#### Na-Acetate Solution:

##### Composition per 10.0mL:

Na-acetate .....	1.6g
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**Preparation of Na-Acetate Solution:** Add Na-acetate to 10.0mL of distilled/deionized water. Mix thoroughly. Filter sterilize.

#### Yeast Extract Peptone Solution:

##### Composition per 10.0mL:

Yeast extract .....	1.0g
Peptone .....	0.5g

#### Preparation of Yeast Extract Peptone Solution:

Add components to 10.0mL of distilled/deionized water. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Aseptically combine 959.0mL basal salts solution, 1.0mL trace elements solution, 10.0mL Na-acetate solution, 10.0mL magnesium calcium solution, 10.0mL yeast extract peptone solution, and 10.0mL biotin solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Sulfitobacter pontiacus*.

#### *Sulfolobus acidocaldarius* Medium (DSMZ Medium 812)

##### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
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KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
Yeast extract .....	0.1g
Glutathione solution .....	10.0mL
pH 2.25 ± 0.1 at 25°C	

#### Glutathione Solution:

##### Composition per 10mL:

Glutathione .....	1.0g
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**Preparation of Glutathione Solution:** Add glutathione to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glutathione solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 2.25. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 10.0mL sterile glutathione solution. Mix thoroughly. Aseptically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Sulfolobus disulfidooxidans*.

#### *Sulfolobus acidocaldarius* Medium

##### Composition 1020.0mL:

Solution A .....	700.0mL
Solution B .....	300.0mL
Solution C .....	20.0mL
pH 1.9–2.4 at 25°C	

#### Solution A:

##### Composition per 700.0mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
KCl .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 700.0mL. Mix thoroughly. Adjust pH to 2.0–2.2 with sulfuric acid. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B:

##### Composition per 300.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	44.2g
H <sub>2</sub> SO <sub>4</sub> , 10N solution .....	1.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 300.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

##### Composition per 20.0mL:

Yeast extract .....	0.2g
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**Preparation of Solution C:** Add yeast extract to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 700.0mL of solution A, 300.0mL of solution B, and 20.0mL of solution C. Aseptically adjust pH to 1.9–2.4

**Use:** For the cultivation of *Sulfolobus thermosulfidooxidans*.

#### *Sulfolobus acidocaldarius* Simplified Basal Medium

##### Composition per liter:

K <sub>2</sub> SO <sub>4</sub> .....	6.0g
NaH <sub>2</sub> PO <sub>4</sub> .....	1.0g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.6g
CaCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.2g
Trace minerals solution .....	0.04mL
pH 3.5 ± 0.2 at 25°C	

**Trace Minerals Solution:****Composition per 100.0mL:**

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	5.0g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.5g
ZnCl <sub>2</sub> .....	0.5g
HCl (1N solution) .....	100.0mL

**Preparation of Trace Minerals Solution:** Combine components. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.5 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Sulfolobus acidocaldarius*.

***Sulfolobus brierleyi* Medium****Composition per liter:**

Sulfur flowers .....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) .....	0.01g
Yeast extract solution .....	20.0mL
pH 1.5–2.5 at 25°C	

**Preparation of Sulfur:** Autoclave sulfur at 8 psi pressure–112°C for 15 min.

**Yeast Extract Solution:****Composition per 20.0mL:**

Yeast extract .....	0.2g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except yeast extract solution and sulfur, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH with 6N H<sub>2</sub>SO<sub>4</sub> to 1.5–2.5. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile yeast extract solution and 10.0g of sterile sulfur. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Acidianus brierleyi*.

***Sulfolobus* Broth****Composition per liter:**

Sucrose yeast solution .....	500.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution .....	250.0mL
Trace elements solution .....	250.0mL
pH 3.0–3.5 at 25°C	

**Sucrose Yeast Solution:****Composition per 500.0mL:**

Sucrose .....	2.0g
Yeast extract .....	1.0g

**Preparation of Sucrose Yeast Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:****Composition per 250.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0g
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**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition per 250.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	28.0mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	4.5mg
MnCl <sub>2</sub> ·7H <sub>2</sub> O .....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 500.0mL of sterile sucrose yeast solution with 250.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution and 250.0mL of sterile trace elements solution. Mix thoroughly. Adjust pH to 3.0–3.5 with sterile H<sub>2</sub>SO<sub>4</sub>. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Sulfolobus* species.

***Sulfolobus* Medium****Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

pH 2.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH at 25°C to 2.0 with 10N H<sub>2</sub>SO<sub>4</sub>. Filter sterilize. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation and maintenance of *Sulfolobus acidocaldarius*.

***Sulfolobus* Medium****Composition per liter:**

Gellan sucrose yeast solution .....	500.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O/MgCl <sub>2</sub> ·6H <sub>2</sub> O solution .....	250.0mL
Trace elements solution .....	250.0mL
pH 3.0–3.5 at 25°C	

**Gellan Sucrose Yeast Solution:****Composition per 500.0mL:**

Gellan gum .....	6.5g
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Sucrose.....	2.0g
Yeast extract.....	1.0g

**Source:** Gellan gum is available from Kelco.

**Preparation of Gellan Sucrose Yeast Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C.

#### CaCl<sub>2</sub>·2H<sub>2</sub>O/MgCl<sub>2</sub>·6H<sub>2</sub>O Solution:

**Composition** per 250.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.44g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.0g

**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O/MgCl<sub>2</sub>·6H<sub>2</sub>O Solution:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C.

#### Trace Elements Solution:

**Composition** per 250.0mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	28.0mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C.

**Preparation of Medium:** Aseptically combine 500.0mL of sterile gellan sucrose yeast solution with 250.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O/MgCl<sub>2</sub>·6H<sub>2</sub>O solution and 250.0mL of sterile trace elements solution. Mix thoroughly. Adjust pH to 3.0–3.5 with sterile H<sub>2</sub>SO<sub>4</sub>. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Sulfolobus* species.

#### *Sulfolobus* Medium, Revised

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Tryptone.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
Yeast extract.....	0.05g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

pH 3.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH at 25°C to 3.0 with 10N H<sub>2</sub>SO<sub>4</sub>. Filter sterilize. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation and maintenance of *Sulfolobus* species.

#### *Sulfolobus shibatae* Medium

**Composition** per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Yeast extract.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> .....	0.01mg

pH 3.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.5 with 10N H<sub>2</sub>SO<sub>4</sub>. Filter sterilize. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation of *Sulfolobus shibatae*.

#### *Sulfolobus solfataricus* Medium

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	3.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.5g
Casamino acids.....	1.0g
Yeast extract.....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01mg

pH 4.0–4.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH at 25°C to 4.0–4.2 with 10N H<sub>2</sub>SO<sub>4</sub>. Filter sterilize. Aseptically distribute into tubes or flasks.

**Use:** For the cultivation and maintenance of *Sulfolobus solfataricus*.

#### *Sulfophobococcus zilligii* Medium (DSMZ Medium 770)

**Composition** per 1035mL:

Glycine.....	1.5g
Na <sub>2</sub> CO <sub>3</sub> .....	230.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	66.0mg
Na <sub>2</sub> -EDTA.....	32.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	31.0mg
KCl.....	31.0mg
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	2.3mg
ZnCl <sub>2</sub> .....	2.1mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	1.8mg
Resazurin.....	0.5mg
Serum albumin solution.....	10.0mL
Dithiothreitol solution.....	10.0mL
Yeast extract solution.....	10.0mL
Iron sulfate solution.....	5.0mL

pH 7.6 ± 0.2 at 25°C

#### Dithiothreitol Solution:

**Composition** per 10mL:

Dithiothreitol.....	1.54mg
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**Preparation of Dithiothreitol Solution:** Add dithiothreitol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Iron Sulfate Solution:**

**Composition per 10mL:**

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g

**Preparation of Iron Sulfate Solution:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Serum Albumin Solution:**

**Composition per 10mL:**

Bovine serum albumin, Fraction V ..... 1.0g

**Preparation of Serum Albumin Solution:** Add bovine serum albumin, Fraction V to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Yeast Extract Solution:**

**Composition per 10.0mL:**

Yeast extract ..... 1.0g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare medium anaerobically under 100% N<sub>2</sub> gas. Add components, except iron sulfate solution, serum albumin solution, dithiothreitol solution and yeast extract solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Cool to 80–90°C. Adjust pH to 7.6. Cool to room temperature. Dispense 30.0mL aliquots into serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically inject per each 30.0mL the following solutions: 0.3mL sterile yeast extract solution, 0.3mL sterile dithiothreitol solution, 0.15mL sterile iron sulfate solution, and 10.0mL sterile serum albumin solution. Final pH should be 7.6.

**Use:** For the cultivation of *Sulfophobococcus zilligii*.

***Sulforhabdus Medium*  
(DSMZ Medium 386a)**

**Composition per 1002mL:**

Solution A ..... 920.0mL  
Solution C ..... 50.0mL  
Solution D ..... 10.0mL  
Solution E ..... 10.0mL  
Solution F ..... 10.0mL  
Solution B  
(Trace elements solution SL-10B) ..... 1.0mL  
pH 7.2–7.5 at 25°C

**Solution A:**

**Composition per 920mL:**

NaCl ..... 1.0g  
KCl ..... 0.5g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.4g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
NH<sub>4</sub>Cl ..... 0.3g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.15g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution B (Trace Elements Solution SL-10B):**

**Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
H<sub>3</sub>BO<sub>3</sub> ..... 300.0mg  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 7.7mL

**Preparation of Solution B (Trace Elements Solution SL-10B):**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C:**

**Composition per 100.0mL:**

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas until saturated, approximately 20 min. Filter sterilize under 100% CO<sub>2</sub> into a sterile, gas-tight 100.0mL screw-capped bottle.

**Solution D:**

**Composition per 10.0mL:**

Na<sub>2</sub>-acetate·3H<sub>2</sub>O ..... 0.3g

**Preparation of Solution D:** Add Na<sub>2</sub>-acetate to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Filter sterilize. Store anaerobically.

**Solution E:**

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution E:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Solution F:**

**Composition per 10.0mL:**

Na-thiosulfate ..... 2.5g

**Preparation of Solution F:** Add Na-thiosulfate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

**Preparation of Medium:** Add solution B, solution C, solution D, solution E, and solution F to solution A in that order under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas. Adjust the pH to 7.2–7.5.

**Use:** For the cultivation of *Desulfocapsa thiozymogenes*.

**Sulfur Medium**

**Composition per liter:**

Sulfur, elemental ..... 10.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 3.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.3g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.25g  
FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 0.02g

pH 4.8± 0.2 at 25°C

**Preparation of Medium:** Add components, except sulfur, to distilled/deionized water and bring volume to 1.0L.

Mix thoroughly. Add 1.0g of sulfur to each of 10 250.0mL flasks. Add 100.0mL of medium to each flask. Autoclave for 30 min at 0 psi pressure–100°C on 3 consecutive days.

**Use:** For the isolation, cultivation, and enumeration of iron and sulfur bacteria.

### *Sulfurospirillum* Medium

#### Composition per 1004.0mL:

Solution A .....	900.0mL
Solution C .....	80.0mL
Solution D .....	20.0mL
Solution B (Trace elements solution SL-10).....	2.0mL
Solution E .....	2.0mL

pH 7.2 ± 0.2 at 25°C

#### Solution A:

##### Composition per 900.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	1.36g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.37g
NH <sub>4</sub> Cl .....	0.27g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B (Trace Elements Solution SL-10):

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

##### Composition per 80.0mL:

NaHCO <sub>3</sub> .....	4.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 80.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution D:

##### Composition per 20.0mL:

Sodium fumarate .....	4.0g
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**Preparation of Solution D:** Add sodium fumarate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E:

##### Composition per 2.0mL:

L-Cysteine-HCl .....	0.063g
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**Preparation of Solution E:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 2.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To sterile solution A in tubes or flasks, add, using a syringe, appropriate volumes of sterile solution B, solution C, solution D, and solution E. Mix thoroughly.

**Use:** For the cultivation of *Sulfurospirillum deleyianum*.

### *Sulfurospirillum* II Medium

#### (DSMZ Medium 771)

##### Composition per 1080mL:

Yeast extract .....	1.0g
NaCl .....	460.0mg
K <sub>2</sub> HPO <sub>4</sub> .....	225.0mg
KH <sub>2</sub> PO <sub>4</sub> .....	225.0mg
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	225.0mg
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	117.0mg
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	30.0mL
NaNO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Na-lactate solution .....	10.0mL
Vitamin solution .....	10.0mL
Cysteine solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
Selenite-tungstate solution .....	1.0mL

pH 7.3 ± 0.2 at 25°C

#### Cysteine Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O .....	0.15g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Selenite-Tungstate Solution

##### Composition per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Na-lactate Solution:

##### Composition per 10.0mL:

Na-lactate .....	2.25g
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**Preparation of Na-lactate Solution:** Add Na-lactate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### NaNO<sub>3</sub> Solution:

##### Composition per 10.0mL:

NaNO <sub>3</sub> .....	1.7g
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**Preparation of NaNO<sub>3</sub> Solution:** Add NaNO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.1g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition** per 30.0mL:

NaHCO <sub>3</sub> .....	4.2g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, cysteine solution, NaNO<sub>3</sub> solution, Na-lactate solution, and vitamin solution, to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 7.3. Dispense either 10.0mL aliquots into 15mL Hungate tubes or 50.0mL aliquots into 100mL Hungate bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically inject from sterile stock solutions NaHCO<sub>3</sub>, Na<sub>2</sub>S·9H<sub>2</sub>O solution, cysteine solution, vitamin solution, sodium nitrate solution, and sodium lactate solution. Final pH should be 7.3.

**Use:** For the cultivation of *Sulfurospirillum arsenophilum* (*Geospirillum* sp.) and *Sulfurospirillum barnesii*.

**Super MMB Medium  
(LMG Medium 188)**

Yeast extract .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
Peptone .....	0.4g
Sodium succinate .....	0.4g
NH <sub>4</sub> Cl .....	0.2g
NaCl .....	0.2g

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	10.0mg
Ferric citrate .....	5.0mg
Vitamin Solution .....	20.0mL
SL-6 Trace Elements Solution.....	1.0mL

pH 7.0 ± 0.2 at 25°C

**SL-6 Trace Elements Solution:****Composition** per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of SL-6 Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Vitamin Solution:****Composition** per liter:

Calcium DL-pantothenate .....	5.0mg
Riboflavin.....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to 980.0mL distilled/deionized water. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 20.0mL sterile vitamin solution. Mix thoroughly. Aseptically distribute to sterile tubes or flasks.

**Use:** For the cultivation of *Aquabacter* spp.

**SWM Medium****Composition** per 1014.0mL:

Solution A .....	940.0mL
Solution E (NaHCO <sub>3</sub> solution).....	50.0mL
Solution F (Substrate solution).....	10.0mL
Solution G (Na <sub>2</sub> S·9H <sub>2</sub> O solution).....	10.0mL
Solution B (Trace elements solution SL-10).....	2.0mL
Solution C (Seven vitamin solution).....	1.0mL
Solution D (Selenite-tungstate solution).....	1.0mL

pH 7.2–7.4 at 25°C

**Solution A:****Composition** per 940.0mL:

NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg

**Preparation of Solution A:** Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Prepare and dispense under 100% N<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C (Seven Vitamin Solution):**

**Composition per liter:**

Pyridoxine·HCl .....	300.0mg
Nicotinic acid .....	200.0mg
Thiamine·HCl .....	200.0mg
Calcium pantothenate .....	100.0mg
Cyanocobalamine .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Solution C (Seven Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Solution D (Selenite-Tungstate Solution):**

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Solution D (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E (NaHCO<sub>3</sub> Solution):**

**Composition per 50.0mL:**

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of Solution E (NaHCO<sub>3</sub> Solution):** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F (Substrate Solution):**

**Composition per 10.0mL:**

Pyrogallol .....	0.5g
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**Preparation of Solution F (Substrate Solution):** Add pyrogallol to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution G (Na<sub>2</sub>S·9H<sub>2</sub>O Solution):** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring vol-

ume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 940.0mL of sterile solution A, aseptically and anaerobically add 1.0mL of sterile solution B, 1.0mL of sterile solution C, 1.0mL of sterile solution D, 50.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Pelobacter massiliensis*.

### **SWMTY Marine Medium**

**Composition per liter:**

Marine salts mix .....	38.0g
Agar .....	15.0g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
Tris(hydroxymethyl)aminomethane buffer .....	1.0g
KNO <sub>3</sub> .....	0.5g
Sodium glycerophosphate .....	0.1g
Trace elements solution HO-LE .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

#### **Trace Elements Solution HO-LE:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.85g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8g
Sodium tartrate .....	1.77g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.36g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.04g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.027g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
ZnCl <sub>2</sub> .....	0.02g

#### **Preparation of Trace Elements Solution HO-LE:**

Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a variety of heterotrophic marine bacterial species.

### **SYC Medium**

**Composition per liter:**

Sucrose .....	10.0g
Pancreatic digest of casein .....	8.0g
Yeast extract .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.3g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Agrobacterium tumefaciens*.

### **Synthetic Seawater Medium**

**Composition per liter:**

NaCl .....	27.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	7.0g
Monosodium glutamate .....	5.0g
Tris(hydroxymethyl)aminomethane buffer .....	2.0g

Glucose .....	1.0g
KCl .....	0.6g
CaCl <sub>2</sub> .....	0.3g
Sodium glycerophosphate .....	0.2g
Vitamin B <sub>12</sub> .....	1.0μg

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Leucothrix mucor*.

### *Syntrophobacter pfennigii* Medium

#### Composition per liter:

NaCl .....	1.0g
Na <sub>2</sub> SO <sub>4</sub> .....	0.7g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
Sodium propionate solution .....	50.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
Seven vitamin solution .....	1.0mL
NaHCO <sub>3</sub> solution .....	variable

pH 7.2–7.4 at 25°C

#### Sodium Propionate Solution:

##### Composition per 50.0mL:

Sodium propionate .....	1.5g
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**Preparation of Sodium Propionate Solution:** Add sodium propionate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 20.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> ·5H <sub>2</sub> O .....	2.0g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg

Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Seven Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	0.3g
Thiamine-HCl .....	0.2g
Nicotinic acid .....	0.2g
Calcium DL-pantothenate .....	0.1g
Vitamin B <sub>12</sub> .....	0.1g
p-Aminobenzoic acid .....	80.0mg
Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare medium and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except sodium propionate solution, NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile sodium propionate solution, 10.0mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically add sufficient volume of sterile NaHCO<sub>3</sub> solution to bring pH to 7.2–7.4. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Syntrophobacter pfennigii*.

### *Syntrophobacter wolinii* Medium

Solution A .....	916.0mL
Solution B .....	70.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL

pH 7.2 ± 0.2 at 25°C

#### Solution A:

##### Composition per 916.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	2.8g
Sodium propionate .....	1.5g
Pancreatic digest of casein .....	1.0g
Resazurin .....	1.0mg
Mineral solution .....	50.0mL
Rumen fluid, clarified .....	50.0mL
Vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Mineral Solution:

## Composition per liter:

Nitrilotriacetic acid	12.5g
NaCl	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
ZnCl <sub>2</sub>	0.1g
CuCl <sub>2</sub>	0.02g
Na <sub>2</sub> SeO <sub>3</sub>	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.017g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g

**Preparation of Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

# Vitamin Solution:

## Composition per liter:

Biotin	0.25mg
Nicotinic acid	2.5mg
Thiamine-HCl	1.25mg
p-Aminobenzoic acid	1.25mg
Pantothenic acid	0.62mg
Pyridoxine-HCl	6.2mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Trace Elements Solution SL-10:

## Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

# Solution B:

## Composition per 70.0mL:

NaHCO <sub>3</sub>	3.5g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

# Solution C:

## Composition per 10.0mL:

L-Cysteine-HCl	0.3g
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**Preparation of Solution C:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

# Solution D:

## Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix

thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Syntrophobacter wolnii*.

# *Syntrophococcus sucromutans* Medium

## Composition per 1002.0mL:

Solution A	916.0mL
Solution C	50.0mL
Solution B	25.0mL
Solution D	10.0mL
Solution E	1.0mL

pH 7.2–7.4 at 25°C

# Solution A:

## Composition per 916.0mL:

Sodium formate	0.6g
Resazurin	1.0mg
Rumen fluid, clarified	300.0mL
Mineral solution	50.0mL
Vitamin solution	5.0mL
Trace elements solution SL-10	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Mix thoroughly. Adjust pH to 6.4. Autoclave for 15 min at 15 psi pressure–121°C.

# Mineral Solution:

## Composition per liter:

KH <sub>2</sub> PO <sub>4</sub>	10.0g
NaCl	8.0g
NH <sub>4</sub> Cl	8.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	6.6g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.0g

**Preparation of Mineral Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Vitamin Solution:

## Composition per liter:

Pyridoxine-HCl	6.2mg
Nicotinic acid	2.5mg
p-Aminobenzoic acid	1.25mg
Thiamine-HCl	1.25mg
Pantothenic acid	0.62mg
Biotin	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Trace Elements Solution SL-10:

## Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L.

Add remaining components. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 25.0mL:

Lactose ..... 5.0g

**Preparation of Solution B:** Add lactose to distilled/deionized water and bring volume to 25.0mL. Mix thoroughly. Filter sterilize.

**Solution C:****Composition** per 50.0mL:

NaHCO<sub>3</sub> ..... 2.5g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition** per 10.0mL:

L-Cysteine ..... 0.24g

**Preparation of Solution D:** Add L-cysteine to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E:****Composition** per 1.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 78.0mg

**Preparation of Solution E:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under H<sub>2</sub>-free 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically combine 916.0mL of sterile solution A with 25.0mL of sterile solution B, 50.0mL of sterile solution C, 10.0mL of sterile solution D, and 1.0mL of sterile solution E. Mix thoroughly. Final pH should be 6.4–6.8

**Use:** For the cultivation and maintenance of *Syntrophococcus sucromutans*.

***Syntrophomonas bryantii* Medium****Composition** per 1026.0mL:

Solution A ..... 916.0mL

Solution B ..... 70.0mL

Solution C ..... 10.0mL

Solution D ..... 10.0mL

Sodium laurate solution ..... 10.0mL

CaCl<sub>2</sub>·2H<sub>2</sub>O solution ..... 10.0mL

pH 7.2 ± 0.2 at 25°C

**Solution A:****Composition** per 916.0mL:

PIPES (Piperazine-*N,N'*-bis  
[2-ethanesulfonic acid]) buffer ..... 15.12g

Na<sub>2</sub>SO<sub>4</sub> ..... 2.8g

Butyric acid ..... 1.7g

Pancreatic digest of casein ..... 1.0g

Resazurin ..... 1.0mg

Mineral solution ..... 50.0mL

Rumen fluid, clarified ..... 50.0mL

Vitamin solution ..... 5.0mL

Trace elements solution SL-10 ..... 1.0mL

**Mineral Solution:****Composition** per liter:

Nitrilotriacetic acid ..... 12.5g

NaCl ..... 1.0g

FeCl<sub>3</sub>·4H<sub>2</sub>O ..... 0.2g

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g

ZnCl<sub>2</sub> ..... 0.1g

CuCl<sub>2</sub> ..... 0.02g

Na<sub>2</sub>SeO<sub>3</sub> ..... 0.02g

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.017g

H<sub>3</sub>BO<sub>3</sub> ..... 0.01g

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl ..... 6.2mg

Nicotinic acid ..... 2.5mg

Thiamine-HCl ..... 1.25mg

*p*-Aminobenzoic acid ..... 1.25mg

Pantothenic acid ..... 0.62mg

Biotin ..... 0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg

MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg

ZnCl<sub>2</sub> ..... 70.0mg

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg

NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg

H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg

CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg

HCl (25% solution) ..... 10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 70.0mL:

NaHCO<sub>3</sub> ..... 3.5g

**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

**Solution C:****Composition** per 10.0mL:

L-Cysteine-HCl ..... 0.3g

**Preparation of Solution C:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix

thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Sodium Laurate Solution:

##### Composition per 10.0mL:

Sodium laurate ..... 2.78g

**Preparation of Sodium Laurate Solution:** Add sodium laurate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:

##### Composition per 40.0mL:

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.84g

**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly. Prior to inoculation, aseptically add 10.0mL of sterile sodium laurate solution and 10.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Syntrophomonas sapovorans*.

### *Syntrophomonas Medium*

#### Composition per 1006.0mL:

Solution A ..... 916.0mL  
Solution B ..... 70.0mL  
Solution C ..... 10.0mL  
Solution D ..... 10.0mL

pH 7.2 ± 0.2 at 25°C

#### Solution A:

##### Composition per 916.0mL:

Na<sub>2</sub>SO<sub>4</sub> ..... 2.8g  
Butyric acid ..... 1.7g  
Pancreatic digest of casein ..... 1.0g  
Resazurin ..... 1.0mg  
Mineral solution ..... 50.0mL  
Rumen fluid, clarified ..... 50.0mL  
Vitamin solution ..... 5.0mL  
Trace elements solution SL-10 ..... 1.0mL

#### Mineral Solution:

##### Composition per liter:

Nitritotriacetic acid ..... 12.5g  
NaCl ..... 1.0g  
FeCl<sub>3</sub>·4H<sub>2</sub>O ..... 0.2g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
ZnCl<sub>2</sub> ..... 0.1g  
CuCl<sub>2</sub> ..... 0.02g  
Na<sub>2</sub>SeO<sub>3</sub> ..... 0.02g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.017g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine·HCl ..... 6.2mg  
Nicotinic acid ..... 2.5mg  
Thiamine·HCl ..... 1.25mg

*p*-Aminobenzoic acid ..... 1.25mg  
Pantothenic acid ..... 0.62mg  
Biotin ..... 0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution SL-10:

##### Composition per liter:

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution B:

##### Composition per 70.0mL:

NaHCO<sub>3</sub> ..... 3.5g

**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

#### Solution C:

##### Composition per 10.0mL:

L-Cysteine·HCl ..... 0.3g

**Preparation of Solution C:** Add L-cysteine·HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

#### Solution D:

##### Composition per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly.

**Use:** For the cultivation of *Syntrophomonas* species.

### *Syntrophomonas Medium, Sulfate-Free*

#### Composition per 1006.0mL:

Solution A ..... 916.0mL  
Solution B ..... 70.0mL  
Solution C ..... 10.0mL  
Solution D ..... 10.0mL

pH 7.2 ± 0.2 at 25°C



**Solution A:****Composition** per 916.0mL:

Butyric acid.....	1.7g
Pancreatic digest of casein.....	1.0g
Resazurin.....	1.0mg
Mineral solution.....	50.0mL
Rumen fluid, clarified.....	50.0mL
Vitamin solution.....	5.0mL
Trace elements solution SL-10.....	1.0mL

**Mineral Solution:****Composition** per liter:

Nitritotriacetic acid.....	12.5g
NaCl.....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O.....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition** per liter:

Pyridoxine·HCl.....	6.2mg
Nicotinic acid.....	2.5mg
Thiamine·HCl.....	1.25mg
<i>p</i> -Aminobenzoic acid.....	1.25mg
Pantothenic acid.....	0.62mg
Biotin.....	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 70.0mL:

NaHCO <sub>3</sub> .....	3.5g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thor-

oughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

**Solution C:****Composition** per 10.0mL:

L-Cysteine·HCl.....	0.3g
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**Preparation of Solution C:** Add L-cysteine·HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly.

**Use:** For the cultivation of *Syntrophus busweldii*.

***Syntrophomonas species Medium*****Composition** per 1006.0mL:

Solution A.....	916.0mL
Solution B.....	70.0mL
Solution C.....	10.0mL
Solution D.....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Solution A:****Composition** per 916.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	2.8g
Pancreatic digest of casein.....	1.0g
Sodium stearate.....	0.61g
Resazurin.....	1.0mg
Mineral solution.....	50.0mL
Rumen fluid, clarified.....	50.0mL
Vitamin solution.....	5.0mL
Trace elements solution SL-10.....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Mineral Solution:****Composition** per liter:

Nitritotriacetic acid.....	12.5g
NaCl.....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O.....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

# **Vitamin Solution:**

## **Composition per liter:**

Pyridoxine-HCl	6.2mg
Nicotinic acid	2.5mg
Thiamine-HCl	1.25mg
<i>p</i> -Aminobenzoic acid	1.25mg
Pantothenic acid	0.62mg
Biotin	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# **Trace Elements Solution SL-10:**

## **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

# **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

# **Solution B:**

## **Composition per 70.0mL:**

NaHCO <sub>3</sub>	3.5g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

# **Solution C:**

## **Composition per 10.0mL:**

L-Cysteine-HCl	0.3g
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**Preparation of Solution C:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

# **Solution D:**

## **Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly.

**Use:** For the cultivation of *Syntrophomonas wolfei*.

## ***Syntrophothermus Medium* (DSMZ Medium 870)**

## **Composition per liter:**

NaHCO <sub>3</sub>	2.5g
NH <sub>4</sub> Cl	0.54g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.15g
KH <sub>2</sub> PO <sub>4</sub>	0.14g
Resazurin	0.5mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
Cysteine solution	10.0mL

Vitamin solution	10.0mL
Substrate solution	10.0mL
Trace elements solution	1.0mL
Selenite-tungstate solution	1.0mL
pH 7.0 ± 0.2 at 25°C	

# **Substrate Solution:**

## **Composition per 10.0mL:**

Na-crotonate	0.86g
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**Preparation of Substrate Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

# **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

## **Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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# **Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

# **Cysteine Solution:**

## **Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# **Selenite-Tungstate Solution:**

## **Composition per liter:**

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

# **Trace Elements Solution:**

## **Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

# **Vitamin Solution:**

## **Composition per liter:**

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg

D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except cysteine solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, and substrate solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 30 min. Distribute into Hungate tubes or serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. For each 10.0mL medium, aseptically and anaerobically add 0.1mL cysteine solution, 0.1mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, and 0.1mL substrate solution. Mix thoroughly.

**Use:** For the cultivation of *Syntrophothermus lipocalidus* DSM 12680.

### *Syntrophus buswellii* II Medium

**Composition** per 1001.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL
Solution F .....	10.0mL
Solution B (Trace elements solution SL-10) .....	1.0mL
pH 7.1–7.4 at 25°C	

#### **Solution A:**

**Composition** per 870.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3–4 min. Allow to cool to room temperature while gassing under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Continue gassing until pH reaches below 6.0. Seal the flask under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B (Trace Elements Solution SL-10):**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thor-

oughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution C:**

**Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Gas under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

#### **Solution D:**

**Composition** per 10.0mL:

Sodium benzoate .....	3.0g
Sodium acetate .....	1.0g

**Preparation of Solution D:** Add sodium propionate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution E (Vitamin Solution):**

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution F:**

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Gas under 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically and anaerobically combine 870.0mL of sterile solution A with 1.0mL of sterile solution B, 100.0mL of sterile solution C, 10.0mL of sterile solution D, 10.0mL of sterile solution E, and 10.0mL of sterile solution F, in that order. Mix thoroughly. Anaerobically distribute into sterile tubes or flasks under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Syntrophus buswellii*.

### *Syntrophus* Medium

**Composition** per 1006.0mL:

Solution A .....	916.0mL
Solution B .....	70.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL

pH 7.2 ± 0.2 at 25°C

#### **Solution A:**

**Composition** per 916.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	2.8g
Sodium benzoate .....	2.0g
Pancreatic digest of casein .....	1.0g
Resazurin .....	1.0mg
Mineral solution .....	50.0mL
Rumen fluid, clarified .....	50.0mL

Vitamin solution.....	5.0mL
Trace elements solution SL-10 .....	1.0mL

# Mineral Solution:

## Composition per liter:

Nitrilotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

# Vitamin Solution:

## Composition per liter:

Pyridoxine-HCl .....	6.2mg
Nicotinic acid .....	2.5mg
Thiamine-HCl .....	1.25mg
<i>p</i> -Aminobenzoic acid .....	1.25mg
Pantothenic acid .....	0.62mg
Biotin .....	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Trace Elements Solution SL-10:

## Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

# Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Solution B:

## Composition per 70.0mL:

NaHCO <sub>3</sub> .....	3.5g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

# Solution C:

## Composition per 10.0mL:

L-Cysteine-HCl .....	0.3g
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**Preparation of Solution C:** Add L-cysteine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

# Solution D:

## Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Syntrophus buswellii*.

# Syntrophus Medium, Sulfate-Free

## Composition per 1006.0mL:

Solution A .....	916.0mL
Solution B .....	70.0mL
Solution C .....	10.0mL
Solution D .....	10.0mL

pH 7.2 ± 0.2 at 25°C

# Solution A:

## Composition per 916.0mL:

Sodium benzoate .....	2.0g
Pancreatic digest of casein .....	1.0g
Resazurin .....	1.0mg
Mineral solution .....	50.0mL
Rumen fluid, clarified .....	50.0mL
Vitamin solution .....	5.0mL
Trace elements solution SL-10 .....	1.0mL

# Mineral Solution:

## Composition per liter:

Nitrilotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

# Vitamin Solution:

## Composition per liter:

Pyridoxine-HCl .....	6.2mg
Nicotinic acid .....	2.5mg
Thiamine-HCl .....	1.25mg
<i>p</i> -Aminobenzoic acid .....	1.25mg
Pantothenic acid .....	0.62mg
Biotin .....	0.25mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 916.0mL. Adjust pH to 7.2. Gently heat and bring to boiling. Continue boiling for a few minutes. Allow to cool to room temperature under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B:****Composition** per 70.0mL:

NaHCO <sub>3</sub> .....	3.5g
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**Preparation of Solution B:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 70.0mL. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min.

**Solution C:****Composition** per 10.0mL:

L-Cysteine·HCl .....	0.3g
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**Preparation of Solution C:** Add L-cysteine·HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Solution D:****Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Solution D:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 3–4 min. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** To 916.0mL of sterile solution A, add 70.0mL of sterile solution B, 10.0mL of sterile solution C, and 10.0mL of sterile solution D. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Syntrophus buswellii*.

**T7 Agar Base  
(m-T7 Agar Base)**

**Composition** per liter:

Lactose .....	20.0g
Agar .....	15.0g
Polyoxyethylene ether W-1 .....	5.0g
Yeast extract .....	3.0g
Pancreatic digest of casein .....	2.5g
Peptic digest of animal tissue .....	2.5g
Sodium heptadecyl sulfate .....	0.1g
Bromothymol Blue .....	0.1g
Bromocresol Purple .....	0.1g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. The medium may be made more selective by adding 1.0mg of penicillin G per liter. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective recovery and differential identification of injured coliform microorganisms from chlorinated water by the membrane filter method. For rapid estimation of the bacteriological quality of water using the membrane filter method.

**T-ASW Medium****Composition** per 1003.0mL:

NaCl .....	25.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	2.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.3g
NaHCO <sub>3</sub> .....	0.2g
Tris·HCl buffer, 0.1M, pH 7.5 .....	200.0mL
Phenol Red (0.5% solution) .....	2.0mL
Trace elements solution .....	1.0mL

pH 7.5 ± 0.2 at 25°C

**Trace Elements Solution:****Composition** per liter:

Disodium EDTA .....	50.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.5g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.2g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.6g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.6g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	1.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 with KOH. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Filter sterilize.

**Use:** For the cultivation and maintenance of *Thiobacillus hydrothermalis*.

**T2 Medium for Thiobacillus****Composition** per liter:

Solution A .....	250.0mL
Solution B .....	250.0mL
Solution C .....	250.0mL
Solution D .....	250.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition** per 250.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
KNO <sub>3</sub> .....	2.0g
NH <sub>4</sub> Cl .....	1.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Filter sterilize.

**Solution B:****Composition** per 250.0mL

KH<sub>2</sub>PO<sub>4</sub> ..... 2.0g

**Preparation of Solution B:** Add KH<sub>2</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Filter sterilize.

**Solution C:****Composition** per 250.0mL

NaHCO<sub>3</sub> ..... 2.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Filter sterilize.

**Solution D:****Composition** per 250.0mL

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.8g

FeSO<sub>4</sub>·7H<sub>2</sub>O (2%, w/v, in 1N HCl) ..... 1.0mL

Trace metal solution ..... 1.0mL

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Filter sterilize.

**FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:****Composition** per 100.0mL

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.0g

HCl (1N solution) ..... 100.0mL

**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add the FeSO<sub>4</sub>·7H<sub>2</sub>O to the HCl solution. Mix thoroughly.

**Trace Metal Solution:****Composition** per liter:

EDTA ..... 50.0g

ZnSO<sub>4</sub> ..... 22.0g

CaCl<sub>2</sub> ..... 5.54g

MnCl<sub>2</sub> ..... 5.06g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 4.99g

CoCl<sub>2</sub> ..... 1.61g

CuSO<sub>4</sub> ..... 1.57g

(NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> ..... 1.1g

**Preparation of Trace Metal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 with KOH.

**Preparation of Medium:** Aseptically combine the four sterile solutions: solution A, solution B, solution C, and solution D. Adjust the pH to 7.0. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiobacillus denitrificans* and other thiobacilli.

**TCBS Agar**  
(Thiosulfate Citrate Bile Salt  
Sucrose Agar)

**Composition** per liter:

Sucrose ..... 20.0g

Agar ..... 14.0g

NaCl ..... 10.0g

Sodium citrate ..... 10.0g

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> ..... 10.0g

Yeast extract ..... 5.0g

Pancreatic digest of casein ..... 5.0g

Peptic digest of animal tissue ..... 5.0g

Oxgall ..... 5.0g

Sodium cholate ..... 3.0g

Ferric citrate ..... 1.0g

Thymol Blue ..... 0.04g

Bromothymol Blue ..... 0.04g

pH 8.6 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Do not autoclave. Cool to 45°–50°C. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the selective isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus* from a variety of nonclinical specimens.

**TCY Agar****Composition** per liter:

NaCl ..... 31.3g

Agar ..... 15.0g

MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 10.8g

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.0g

Casamino acids ..... 1.0g

Tryptone ..... 1.0g

KCl ..... 0.7g

Yeast extract ..... 0.2g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Flexibacter maritimus*.

**TDC Medium****Composition** per liter:

Agar ..... 20.0g

CaCO<sub>3</sub> ..... 10.0g

Glucose ..... 5.0g

K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g

MgSO<sub>4</sub> ..... 1.0g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azotobacter beijerinckii* and other *Azotobacter* species.

**TEC Agar**  
(m-TEC Agar)

**Composition** per liter:

Agar ..... 15.0g

Lactose ..... 10.0g

NaCl ..... 7.5g

Proteose peptone ..... 5.0g

K<sub>2</sub>HPO<sub>4</sub> ..... 3.3g

Yeast extract ..... 3.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g

Sodium lauryl sulfate ..... 0.2g

Sodium deoxycholate ..... 0.1g

Bromocresol Purple ..... 0.08g

Bromphenol Red ..... 0.08g

pH 5.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 5.0. Sterilization is unnecessary. Pour into sterile Petri dishes or distribute into sterile tubes or flasks. Store at 2°–8°C. Use within 1 week.

**Use:** For detection of *Escherichia coli* in recreational waters by the membrane filter method. This agar is used in conjunction with a urea substrate to detect urease production. After addition of the urea substrate, *Escherichia coli* appears as yellow-yellow/brown colonies when viewed under a fluorescent lamp.

#### **Teepol Broth, Enriched (m-Teepol Broth, Enriched)**

**Composition per liter:**

Peptone.....	40.0g
Lactose.....	30.0g
Yeast extract.....	6.0g
Phenol Red.....	0.2g
Sodium lauryl sulfate (Teepol, 0.1% solution).....	4.0mL
pH 7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enumeration of coliform organisms and *Escherichia coli* in water by the membrane filter method.

#### **Tergitol 7 Agar**

**Composition per liter:**

Lactose.....	20.0g
Agar.....	13.0g
Peptone.....	10.0g
Yeast extract.....	6.0g
Meat extract.....	5.0g
Tergitol-7.....	0.1g
Bromothymol Blue.....	0.05g
TTC solution.....	5.0mL
pH 7.2 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

#### **TTC Solution:**

**Composition per 100.0mL:**

Triphenyltetrazolium chloride.....	0.05g
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**Preparation of TTC Solution:** Add triphenyltetrazolium chloride to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 995.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 5.0mL of sterile TTC solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the detection and enumeration of coliforms. Lactose-fermenting bacteria appear as yellow colonies. Lactose-nonfermenting bacteria appear as blue colonies.

#### **Termitobacter Medium**

**Composition per 1060.0mL**

NH <sub>4</sub> Cl.....	1.0g
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NaCl.....	0.6g
Sodium acetate·3H <sub>2</sub> O.....	0.5g
L-Cysteine·HCl.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
KCl.....	0.1g
Yeast extract.....	0.2g
Resazurin.....	0.5mg
NaHCO <sub>3</sub> .....	40.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O.....	10.0mL
Trans-3,4,5-trimethoxycinnamate solution.....	10.0mL
Trace elements solution SL-10.....	1.5mL

#### **NaHCO<sub>3</sub> Solution:**

**Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### **Trans-3,4,5-Trimethoxycinnamate Solution:**

**Composition per 10.0mL:**

Trans-3,4,5-trimethoxycinnamate.....	1.19g
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#### **Preparation of Trans-3,4,5-Trimethoxycinnamate**

**Solution:** Add trans-3,4,5-trimethoxycinnamate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Neutralize with NaOH. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except NaHCO<sub>3</sub> solution, trans-3,4,5-trimethoxycinnamate solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 40.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile trans-3,4,5-trimethoxycinnamate solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Sporobacter termitidis*.

#### **Tetramethyl Ammonium Chloride Agar**

**Composition per liter:**

Agar.....	15.0g
Tetramethyl ammonium chloride.....	1.0g
Thiamine·HCl.....	0.5g
Standard mineral base.....	1.0L
pH 6.5 ± 0.2 at 25°C	

#### **Standard Mineral Base:**

**Composition per liter:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Phosphate buffer (1M solution, pH 6.8).....	40.0mL
Huntner's vitamin-free mineral base.....	20.0mL

**Huntner's Vitamin-Free Mineral Base:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	14.45g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
"Metals 44" .....	50.0mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg

**Preparation of Huntner's Vitamin-Free Mineral**

**Base:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

**"Metals 44":****Composition per 100.0mL:**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1095.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	500.0mg
Ethylenediaminetetraacetic acid .....	250.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	154.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Paracoccus kocurii*, a tetramethyl assimilating bacterium from sewage sludge.

**Tetrathionate Broth****Composition per liter:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	40.7g
CaCO <sub>3</sub> .....	25.0g
NaCl .....	4.5g
Peptone .....	4.5g
Yeast extract .....	1.8g
Beef extract .....	0.9g
Iodine solution .....	20.0mL

**Iodine Solution:****Composition per 20.0mL:**

Iodine .....	6.0g
KI .....	5.0g

**Preparation of Iodine Solution:** Add iodine and KI to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except iodine solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Cool to 40°C. Add 20.0mL of iodine solution. Mix thoroughly. Distribute into tubes in 10.0mL volumes. Use medium the same day it is prepared.

**Use:** For the selective isolation and enrichment of *Salmonella typhi* and other salmonellae from sewage, and other specimens.

**Tetrathionate Reductase Medium****Composition per tube:**

Solution I .....	10.0mL
Solution III .....	0.2mL

Solution II .....	0.1mL
Solution IV .....	0.1mL

**Solution I:****Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	3.6g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
Peptone .....	0.25g
Yeast extract .....	0.25g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.03g

**Preparation of Solution I:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 10.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution II:****Composition per 100.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Ferric ammonium citrate .....	0.05g

**Preparation of Solution II:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat until dissolved. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution III:****Composition per 100.0mL:**

Sodium succinate .....	15.0g
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**Preparation of Solution III:** Add sodium succinate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat until dissolved. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Solution IV:****Composition per 100.0mL:**

Na <sub>2</sub> S <sub>4</sub> O <sub>6</sub> ·2H <sub>2</sub> O .....	10.0g
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**Preparation of Solution IV:** Add Na<sub>2</sub>S<sub>4</sub>O<sub>6</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sterilize by filtration. Store at 4°C.

**Preparation of Medium:** To each tube containing 10.0mL of sterile solution I, aseptically add 0.1mL of sterile solution II, 0.2mL of sterile solution III, and 0.1mL of sterile solution IV. Mix thoroughly. Use immediately.

**Use:** For the cultivation and differentiation of hydrogen-oxidizing bacteria based on their production of tetrathionate reductase.

**TF Medium****Composition per liter:**

NaCl .....	7.0g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.8g
K <sub>2</sub> HPO <sub>4</sub> .....	1.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.4g
Na <sub>2</sub> HPO <sub>4</sub> ·H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.16g
Resazurin .....	0.5mg
Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Glucose solution .....	10.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution .....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution .....	10.0mL

pH 6.8–7.0 at 25°C



**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Glucose Solution:****Composition per 10.0mL:**

D-Glucose .....	3.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:****Composition per 10.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
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**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add 0.06g of CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**L-Cysteine·HCl·H<sub>2</sub>O Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.3g
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**Preparation of L-Cysteine·HCl·H<sub>2</sub>O Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except glucose solution, CaCl<sub>2</sub>·2H<sub>2</sub>O solution, and L-cysteine·HCl·H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 6.8–7.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile glucose solu-

tion, 10.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution, and 10.0mL of sterile L-cysteine·HCl·H<sub>2</sub>O solution. Mix thoroughly. Final pH should be 6.8–7.0.

**Use:** For the cultivation of *Thermosipho* species.

**TF(A) Medium**  
(DSMZ Medium 740)

**Composition per liter:**

K <sub>2</sub> HPO <sub>4</sub> .....	1.6g
Yeast extract .....	2.0g
Trypticase .....	2.0g
Na <sub>2</sub> HPO <sub>4</sub> ·H <sub>2</sub> O .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.16g
Resazurin .....	0.5mg
Calcium chloride solution .....	10.0mL
Glucose solution .....	10.0mL
Cysteine solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL

pH 6.8 ± 0.2 at 25°C

**Calcium Chloride Solution:****Composition per 10.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.06g
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**Preparation of Calcium Chloride Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine·HCl·H <sub>2</sub> O .....	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Glucose Solution:****Composition per 10mL:**

Glucose .....	3.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitritotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g

Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly. Filter sterilize.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub>. Add components, except vitamin solution, cysteine solution, calcium chloride solution, glucose solution, trace elements solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL sterile vitamin solution, 10.0mL of sterile cysteine solution, 10.0mL sterile glucose solution, 10.0mL sterile calcium chloride solution, 10.0mL sterile trace elements solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 6.8. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Fervidobacterium pennivorans*, *Fervidobacterium gondwanense*, and *Fervidobacterium* sp.

#### TGE Broth

##### Composition per liter:

Pancreatic digest of casein .....	10.0g
Beef extract .....	6.0g
Glucose .....	2.0g

pH 7.0 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the enumeration of bacteria by the membrane filter method.

#### *Thauera aromatica* AR-1 Medium (DSMZ Medium 855)

##### Composition per 992.0mL:

Solution A .....	870.0mL
Solution C .....	100.0mL
Solution D .....	10.0mL
Solution E (Vitamin solution) .....	10.0mL

Solution B (Trace elements solution SL-10) .....	1.0mL
Selenite-tungstate solution .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### Solution A:

##### Composition per 870.0mL:

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
NaCl .....	1.0g
KNO <sub>3</sub> .....	0.6g
KCl .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

#### Solution B (Trace Elements Solution SL-10):

##### Composition per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution C:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

#### Solution D:

##### Composition per 10.0mL:

Na-benzoate .....	0.7g
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**Preparation of Solution D:** Add Na-benzoate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

##### Composition per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.10mg

**Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix

thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Selenite-Tungstate Solution:

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, and 1.0mL sterile selenite-tungstate solution. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels. Addition of 10–20mg sodium dithionite per liter from a 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized, may stimulate growth.

**Use:** For the anaerobic cultivation of *Thauera aromatica*.

### *Thauera mechernichi* Medium (DSMZ Medium 918)

**Composition** per liter:

Na <sub>2</sub> HPO <sub>4</sub> .....	4.20g
Na-acetate .....	2.93g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Trace elements solution .....	2.0mL
pH 7.1 ± 0.2 at 25°C	

### Trace Elements Solution:

**Composition** per liter:

Na <sub>2</sub> -EDTA .....	50.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.5g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	1.61g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	1.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thauera mechernichensis*.

### *Thermoacetogenium phaeum* Medium (DSMZ Medium 880)

**Composition** per liter:

KHCO <sub>3</sub> .....	3.5g
NH <sub>4</sub> Cl .....	1.0g
NaCl .....	0.6g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.08g
Resazurin .....	0.5mg

Sodium pyruvate solution .....	50.0mL
Vitamin solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Cysteine solution .....	10.0mL
Trace elements solution .....	1.0mL
Selenite-tungstate solution .....	1.0mL
pH 7.0–7.1 at 25°C	

### Sodium Pyruvate Solution:

**Composition** per 50.0mL:

Sodium pyruvate .....	5.0g
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**Preparation of Sodium Pyruvate Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Selenite-Tungstate Solution:

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Cysteine Solution:

**Composition** per 10.0mL:

L-Cysteine·HCl·H <sub>2</sub> O .....	0.3g
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**Preparation of Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
Na <sub>2</sub> -EDTA .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Adjust pH to 6.5. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg

Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except KHCO<sub>3</sub>, sodium pyruvate solution, cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 10 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add 3.5g KHCO<sub>3</sub>. Mix thoroughly while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL sodium pyruvate solution, 10.0mL cysteine solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Final pH is 7.0–7.1. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermoacetogenium phaeum*.

#### *Thermoactinopolyspora* Medium

**Composition** per liter:

Maltose.....	20.0g
Agar .....	15.0g
Papaic digest of soybean meal.....	15.0g
Yeast extract.....	2.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermoactinomyces* and *Thermoactinopolyspora* species.

#### *Thermoanaerobacter ethanolicus* Medium

**Composition** per liter:

Glucose .....	8.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	4.2g
Yeast extract.....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.18g
Reducing solution .....	40.0mL
Wolfe's modified mineral solution.....	5.0mL
Resazurin (0.1% solution).....	1.0mL
Vitamin solution.....	0.5mL

**Caution:** This medium contains Na<sub>2</sub>S, and H<sub>2</sub>S production will occur, especially upon prolonged boiling. H<sub>2</sub>S is hazardous and preparation of this medium should be done in a chemical fume hood.

**Reducing Solution:**

**Composition** per 200.0mL:

Cysteine·HCl·H <sub>2</sub> O.....	2.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	2.5g
NaOH (0.2N solution).....	200.0mL

**Preparation of Reducing Solution:** Gently heat the NaOH solution and bring to boiling. Gas with 95% N<sub>2</sub> + 5% H<sub>2</sub>. Cool to room temperature. Add the cysteine·HCl·H<sub>2</sub>O

and Na<sub>2</sub>S·9H<sub>2</sub>O. Anaerobically distribute into tubes. Cap with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:**

**Composition** per 500.0mL:

Pyridoxine·HCl.....	0.1g
p-Aminobenzoic acid .....	0.05g
Calcium pantothenate .....	0.05g
Nicotinic acid .....	0.05g
Thioctic acid.....	0.05g
Biotin.....	0.02g
Folic acid.....	0.02g
Riboflavin.....	5.0mg
Thiamine·HCl.....	5.0mg
Vitamin B <sub>12</sub> .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Store solution in the dark at –10°C.

**Wolfe's Modified Mineral Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> (anhydrous).....	0.1g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> (anhydrous) .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> (anhydrous) .....	1.0mg

**Preparation of Wolfe's Modified Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except reducing solution, to distilled/deionized water and bring volume to 1.0L. Gently heat and bring to boiling under 95% N<sub>2</sub> + 5% H<sub>2</sub>. Continue boiling until color changes from blue to pink. Add the reducing solution. The pink color will disappear, indicating that the solution has been reduced. Distribute into tubes or flasks under 95% N<sub>2</sub> + 5% H<sub>2</sub> using anaerobic techniques. Cap tubes with rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of thermophilic anaerobes such as *Thermoanaerobacter* species and some *Clostridium* species.

#### *Thermoanaerobacter subterraneus* Medium (DSMZ Medium 899)

**Composition** per liter:

Yeast extract .....	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
NaCl .....	0.6g
Cysteine·HCl·H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
KCl .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
Resazurin .....	0.5mg
D-Glucose solution .....	30.0mL

NaHCO <sub>3</sub> solution .....	20.0mL
Trace mineral solution .....	10.0mL
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL
pH 7.0 ± 0.2 at 25°C	

**Na<sub>2</sub>S-9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S-9H <sub>2</sub> O .....	0.45g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	4.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Glucose Solution:****Composition per 30.0mL:**

Glucose .....	4.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 30.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:****Composition per 10mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	2.5g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, glucose solution, Na<sub>2</sub>S-9H<sub>2</sub>O solution, and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, to distilled/deionized water and bring volume to 930.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Distribute into sterile tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically per 1.0L of medium add 20.0mL NaHCO<sub>3</sub> solution, 30.0mL glucose solution, 10.0mL Na<sub>2</sub>S-9H<sub>2</sub>O solu-

tion, and 10.0mL Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Thermoanaerobacter subterraneus*.

***Thermoanaerobacter sulfurophilus* Medium  
(DSMZ Medium 827)**

**Composition per 1055mL:**

Sulfur, powdered .....	10.0g
NH <sub>4</sub> Cl .....	0.33g
KCl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
Resazurin .....	0.5mg
Glucose solution .....	25.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	15.0mL
Vitamin solution .....	10.0mL
Yeast extract solution .....	5.0mL
Trace elements solution SL10 .....	1.0mL
pH 7.0 ± 0.2 at 25°C	

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Glucose Solution:****Composition per 50mL:**

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Yeast Extract Solution:****Composition per 10mL:**

Yeast extract .....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L.

Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 20mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.6g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, glucose solution, vitamin solution, and yeast extract solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Heat to 90°C on each of 3 successive days. Aseptically and anaerobically add 25.0mL sterile glucose solution, 15.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL sterile vitamin solution, and 5.0mL sterile yeast extract solution. Mix thoroughly. Aseptically and anaerobically distribute into tubes or bottles. The pH should be 7.0.

**Use:** For the cultivation of *Thermoanaerobacter sulfophilus*.

#### *Thermoanaerobacter tengcongensis* Medium (DSMZ Medium 965)

**Composition** per liter:

Soluble Starch .....	10.0g
NaCl .....	2.0g
Tryptone .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Yeast extract .....	1.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Cysteine·HCl·H <sub>2</sub> O .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
KCl .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Resazurin .....	0.5mg
Thiosulfate solution .....	50.0mL
Trace elements solution .....	10.0mL

pH 7.5 ± 0.2 at 25°C

#### Thiosulfate Solution:

**Composition** per 50mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 5.0g

**Preparation of Thiosulfate Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except thiosulfate solution and cysteine·HCl·H<sub>2</sub>O, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to room temperature while sparging with 100% N<sub>2</sub> gas. Adjust pH to 7.5. Distribute into tubes or bottles under 100% N<sub>2</sub> gas. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically under 100% N<sub>2</sub> gas, add 50.0mL sterile thiosulfate solution per liter of medium.

**Use:** For the cultivation of *Thermoanaerobacter tengcongensis*.

#### *Thermoanaerobacterium* Medium (DSMZ Medium 903)

**Composition** per 1030mL:

KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
NaCl .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
Trypticase .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Resazurin .....	0.5mg
Sucrose solution .....	50.0mL
NaHCO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
L-Cysteine solution .....	10.0mL
Selenite solution .....	1.0mL
Seven vitamin solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Sucrose Solution:

**Composition** per 50.0mL:

Sucrose ..... 5.0g

**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### L-Cysteine Solution:

**Composition** per 10.0mL:

L-Cysteine·HCl·H<sub>2</sub>O ..... 0.3g

**Preparation of L-Cysteine Solution:** Add L-cysteine·HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.3g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### NaHCO<sub>3</sub> Solution:

**Composition** per 20.0mL:

NaHCO<sub>3</sub> ..... 2.5g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix

thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

#### Selenite Solution:

**Composition** per liter:

NaOH .....	0.5g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Seven Vitamin Solution:

**Composition** per liter:

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
<i>p</i> -Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except seven vitamin solution, NaHCO<sub>3</sub> solution, sucrose solution, L-cysteine-HCl-H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Adjust pH to 7.0. Distribute into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter, 10.0mL seven vitamin solution, 20.0mL NaHCO<sub>3</sub> solution, 50.0mL sucrose solution, 10.0mL L-cysteine-HCl-H<sub>2</sub>O solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O. Mix thoroughly. The final pH should be 7.0.

**Use:** For the cultivation of *Thermoanaerobacterium polysaccharolyticum* and *Thermoanaerobacterium zeae*.

#### *Thermoanaerobium brockii* Medium

**Composition** per liter:

Pancreatic digest of casein .....	10.0g
Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.9g
NaCl .....	0.9g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Glucose solution .....	25.0mL

Na <sub>2</sub> S·9H <sub>2</sub> O (10% solution) .....	10.0mL
Trace elements solution .....	9.0mL
Vitamin solution .....	5.0mL
Resazurin (0.025% solution) .....	4.0mL
FeSO <sub>4</sub> ·7H <sub>2</sub> O (10% solution) .....	0.03mL
pH 7.3 ± 0.2 at 25°C	

#### Glucose Solution:

**Composition** per 100.0mL:

Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution:

**Composition** per liter:

Nitrilotriacetic acid .....	12.5g
NaCl .....	1.0g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	0.2g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.017g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### Wolfe's Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
Calcium pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Thioctic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Cyanocobalamin .....	100.0μg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 975.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. While still hot, aseptically add 25.0mL of the sterile glucose solution under 97% N<sub>2</sub> + 3% H<sub>2</sub>. Adjust pH to 7.3 if necessary. Aseptically and anaerobically distribute into tubes. Cap with rubber stoppers.

**Use:** For the cultivation and maintenance of *Thermoanaerobium brockii*.

#### *Thermoanaeromonas* Medium (DSMZ Medium 963)

**Composition** per liter:

NaHCO <sub>3</sub> .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.78g
KH <sub>2</sub> PO <sub>4</sub> .....	0.75g
NH <sub>4</sub> Cl .....	0.5g
Glucose .....	0.5g
Yeast extract .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g

NaCl .....	0.2g
Na <sub>3</sub> EDTA .....	0.04g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.03g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Resazurin .....	0.5mg
Thiosulfate solution .....	20.0mL
Vitamin solution .....	10.0mL
Trace elements solution .....	10.0mL
Cysteine solution .....	10.0mL

pH 6.5 ± 0.2 at 25°C

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Cysteine Solution:****Composition per 10.0mL:**

L-Cysteine-HCl·H <sub>2</sub> O .....	0.25g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Thiosulfate Solution:****Composition per 20mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	1.24g
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**Preparation of Thiosulfate Solution:** Add 1.24g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub>, vitamin solution, thiosulfate solution, and cysteine solution, to distilled/deionized water and bring vol-

ume to 960.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add solid NaHCO<sub>3</sub>. Adjust pH to 6.8–7.0. Distribute into anaerobe tubes or bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium, 10.0mL sterile vitamin solution, 10.0mL sterile cysteine solution, and 20.0mL sterile thiosulfate solution. Mix thoroughly. The final pH should be 6.5.

**Use:** For the cultivation of *Thermoanaeromonas toyohensis*.

### **Thermoanaerovibrio Medium (DSMZ Medium 873)**

**Composition per liter:**

NaHCO <sub>3</sub> .....	0.8g
NH <sub>4</sub> Cl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.22g
KCl .....	0.33g
Yeast extract .....	0.25g
Peptone .....	0.25g
Resazurin .....	0.5mg
NaHCO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	10.0mL
Glucose solution .....	10.0mL
Calcium chloride solution .....	10.0mL
Magnesium chloride solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.0–7.3 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Magnesium Chloride Solution:****Composition per 10.0mL:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
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**Preparation of Magnesium Chloride Solution:**

Add MgCl<sub>2</sub>·6H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Calcium Chloride Solution:****Composition per 10.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.22g
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**Preparation of Calcium Chloride Solution:**

Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Glucose Solution:****Composition per 10.0mL:**

Glucose .....	3.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

#### **Vitamin Solution:**

##### **Composition per liter:**

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### **Trace Elements Solution SL-10:**

##### **Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	100.0mg
ZnCl <sub>2</sub>	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O	24.0mg
H <sub>3</sub> BO <sub>3</sub>	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	2.0mg
HCl (25% solution)	10.0mL

**Preparation of Trace Elements Solution SL-10:** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas atmosphere. Add components, except NaHCO<sub>3</sub> solution, glucose solution, calcium chloride solution, magnesium chloride solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, vitamin solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 929.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL glucose solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL magnesium chloride solution, 10.0mL calcium chloride solution, 10.0mL vitamin solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Adjust pH to 7.0–7.3 with 10.0mL NaHCO<sub>3</sub> solution. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermoanaerobivrio velox* (*Thermosinus velox*).

#### **Thermobacterium Medium**

##### **Composition per liter:**

Agar	20.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.3g
Yeast extract	1.0g
Pancreatic digest of casein	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.247g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.074g

FeCl <sub>3</sub> ·6H <sub>2</sub> O	0.019g
Salt solution	1.0mL
pH 8.5 + 0.2 at 25°C	

#### **Salt Solution:**

##### **Composition per liter:**

Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O	4.4g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.8g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22g
CuCl <sub>2</sub> ·H <sub>2</sub> O	0.05g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.03g
VOSO <sub>4</sub> ·2H <sub>2</sub> O	0.03g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2.0 with H<sub>2</sub>SO<sub>4</sub>.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes in 11.0–12.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to solidify in a slanted position.

**Use:** For the cultivation and maintenance of *Thermomicrobium roseum*.

#### **Thermobacteroides leptospartum Medium**

##### **Composition per 1168.1mL:**

Yeast extract	2.0g
Trypticase	2.0g
NaOH solution	1.0L
Glucose solution	113.0mL
Na <sub>2</sub> S solution	22.6mL
Solution A	10.0mL
Mineral salts solution	10.0mL
Solution B	2.0mL
Resazurin solution	0.5mL

#### **NaOH Solution:**

##### **Composition per liter:**

NaOH	4.0g
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**Preparation of NaOH Solution:** Add NaOH to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Glucose Solution:**

##### **Composition per 100.0mL:**

D-Glucose	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 15 min. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Na<sub>2</sub>S Solution:**

Na <sub>2</sub> S	2.5g
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**Preparation of Na<sub>2</sub>S Solution:** Gently heat 100.0mL of distilled/deionized water to 100°C. Boil for 5 min. Sparge with 100% N<sub>2</sub> for 15 min. Add the Na<sub>2</sub>S. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 10 min. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution A:**

##### **Composition per liter:**

NH <sub>4</sub> Cl	100.0g
MgCl <sub>2</sub> ·H <sub>2</sub> O	100.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	40.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4 with HCl.

# Mineral Salts Solution:

## Composition per liter:

EDTA·2H <sub>2</sub> O	0.5g
CoCl <sub>2</sub> ·H <sub>2</sub> O	0.15g
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
ZnCl <sub>2</sub>	0.1g
AlCl <sub>3</sub> ·H <sub>2</sub> O	40.0mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	30.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	20.0mg
NiSO <sub>4</sub> ·H <sub>2</sub> O	20.0mg
H <sub>2</sub> SeO <sub>3</sub>	10.0mg
H <sub>3</sub> BO <sub>4</sub>	10.0mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O	10.0mg

**Preparation of Mineral Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3 with HCl.

# Solution B:

## Composition per liter:

K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	200.0g
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**Preparation of Solution B:** Add K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

# Resazurin Solution:

## Composition per 100.0mL:

Resazurin	0.2g
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**Preparation of Resazurin Solution:** Add resazurin to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Sparge 1.0L of NaOH solution with 100% CO<sub>2</sub> for 30 min. Add 2.0g of yeast extract and 2.0g of Trypticase. Mix thoroughly. Add 10.0mL of solution A, 2.0mL of solution B, 0.5mL of resazurin solution, and 10.0mL of mineral salts solution with pipets which have been flushed a few times with 100% N<sub>2</sub>. Mix thoroughly. Anaerobically distribute 9.0mL volumes into anaerobic tubes fitted with butyl rubber stoppers. Autoclave for 15 min at 15 psi pressure–121°C. One hr prior to inoculation, add 1.0mL of sterile glucose solution and 0.2mL of sterile Na<sub>2</sub>S solution to each 9.0mL of medium.

**Use:** For the cultivation of *Thermobacteroides leptospartum*.

# *Thermobacteroides proteolyticus* Medium

## Composition per 1010.0mL:

NaHCO <sub>3</sub>	5.0g
Pancreatic digest of casein	2.0g
Yeast extract	2.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	1.0g
NH <sub>4</sub> Cl	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.4g
K <sub>2</sub> HPO <sub>4</sub>	0.4g
Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
Resazurin	1.0mg
Trace elements solution	10.0mL

pH 7.0 ± 0.2 at 25°C

# Trace Elements Solution:

## Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g

CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thermobacteroides proteolyticus*.

# *Thermococcus celer* Medium

## Composition per liter:

NaCl	40.0g
Sulfur	10.0g
Yeast extract	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.3g
KH <sub>2</sub> PO <sub>4</sub>	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.07g
FeCl <sub>2</sub> ·2H <sub>2</sub> O	0.02g
NaB <sub>4</sub> O <sub>4</sub> ·10H <sub>2</sub> O	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.8mg
Resazurin	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.05mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O	0.03mg
CoSO <sub>4</sub>	0.01mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL

pH 5.8 ± 0.2 at 25°C

# Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

## Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.8. Do not autoclave. Sterilize by steaming at 100°C for 30 min on 3 consecutive days. Before inoculation, add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Thermococcus celer*.

# *Thermococcus chitinophagus* Medium (DSMZ Medium 766)

## Composition per liter:

Chitin, purified	4.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.5g
KH <sub>2</sub> PO <sub>4</sub>	0.5g
Resazurin	1.0mg
(NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> ) <sub>2</sub>	0.3mg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	0.15mg
Na <sub>2</sub> SeO <sub>4</sub>	0.15mg

Synthetic seawater .....	485.0mL
Trace elements SL-6 .....	15.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL
NaHCO <sub>3</sub> solution .....	10.0mL
pH 6.7 ± 0.2 at 25°C	

**Na<sub>2</sub>S-9H<sub>2</sub>O Solution:****Composition per 10mL:**

Na <sub>2</sub> S-9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	0.2g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Trace Elements Solution SL-6:****Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Synthetic Seawater:****Composition per liter:**

NaCl .....	23.477g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.981g
Na <sub>2</sub> SO <sub>4</sub> .....	3.917g
CaCl <sub>2</sub> .....	1.12g
KCl .....	664.0mg
NaHCO <sub>3</sub> .....	192.0mg
H <sub>3</sub> BO <sub>3</sub> .....	26.0mg
SrCl <sub>2</sub> .....	24.0mg
KBr .....	6.0mg
NaF .....	3.0mg

**Preparation of Synthetic Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Purified Chitin:** Cool 200.0mL of 37% HCl to 4°C. Add 20.0g chitin (practical grade from crab shells) to the cooled HCl. Mix thoroughly. Stir for 60 min at 4°C. Pour the suspension into 1 liter of distilled water, precooled to 4°C. Filter through filter paper. Wash the residue 5 times with 500mL distilled water. Resuspend in 1L of distilled water. Neutralize the suspension with 10mL of 5M KOH to achieve a final pH 6.5. Filter and wash with 3L of distilled water to remove KCl. Allow to air dry.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution and Na<sub>2</sub>S-9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 980.0mL. Gently heat and bring to boiling. Boil for 5 min. Cool to 25°C while sparging with 100% N<sub>2</sub>. Add 10.0mL NaHCO<sub>3</sub> solution. Adjust pH to 6.7. Distribute the medium into Hungate tubes

under an atmosphere of 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Reduce the medium by adding 10.0mL Na<sub>2</sub>S-9H<sub>2</sub>O solution. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermococcus chitinophagus*.

***Thermococcus litoralis* Medium****Composition per liter:**

NaCl .....	25.0g
Sulfur .....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Yeast extract .....	1.0g
Peptone .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.02g
NaB <sub>4</sub> O <sub>4</sub> ·10H <sub>2</sub> O .....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8mg
Resazurin .....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> .....	0.01mg
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

**Na<sub>2</sub>S-9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S-9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S-9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Do not autoclave. Sterilize by steaming at 100°C for 30 min on 3 consecutive days. Before inoculation, add 10.0mL of sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Thermococcus litoralis*.

***Thermococcus* Medium  
(DSMZ Medium 806)****Composition per liter:**

NaCl .....	18.0g
Sulfur .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.4g
MgCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.7g
Yeast extract .....	1.0g
Trypticase .....	1.0g
NaHCO <sub>3</sub> .....	1.0g
KCl .....	0.33g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Resazurin .....	0.001g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.001mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.001mg
Trace elements solution .....	10.0mL
Vitamin solution .....	10.0mL
Cysteine solution .....	10.0mL
Na <sub>2</sub> S-9H <sub>2</sub> O solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

# **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

## **Composition per 10mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O.....0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to 7.0.

# **Cysteine Solution:**

## **Composition per 10.0mL:**

L-Cysteine-HCl·H<sub>2</sub>O.....0.3g

**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

# **Trace Elements Solution:**

## **Composition per liter:**

MgSO<sub>4</sub>·7H<sub>2</sub>O.....3.0g  
Nitrilotriacetic acid.....1.5g  
NaCl.....1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O.....0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O.....0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O.....0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O.....0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O.....0.1g  
NiCl<sub>2</sub>·6H<sub>2</sub>O.....0.025g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O.....0.02g  
H<sub>3</sub>BO<sub>3</sub>.....0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O.....0.01g  
CuSO<sub>4</sub>·5H<sub>2</sub>O.....0.01g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O.....0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

# **Vitamin Solution:**

## **Composition per liter:**

Pyridoxine-HCl.....10.0mg  
Thiamine-HCl·2H<sub>2</sub>O.....5.0mg  
Riboflavin.....5.0mg  
Nicotinic acid.....5.0mg  
D-Ca-pantothenate.....5.0mg  
p-Aminobenzoic acid.....5.0mg  
Lipoic acid.....5.0mg  
Biotin.....2.0mg  
Folic acid.....2.0mg  
Vitamin B<sub>12</sub>.....0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except Fildes enrichment solution, NaHCO<sub>3</sub>, vitamin solution, cysteine solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 5 min. Cool to room temperature under 100% N<sub>2</sub>. Add 1.0g solid NaHCO<sub>3</sub>. Adjust pH to 7.2. Sterilize at 100°C for 3 h on 3 consecutive days. Aseptically and anaerobically add 10.0mL vitamin solution, 10.0mL cysteine solution, and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Adjust pH to 7.2. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermococcus* spp.

# ***Thermococcus profundus* Medium**

## **Composition per 1010.0mL:**

NaCl.....25.0g  
Sulfur.....10.0g  
Peptone.....5.0g  
Yeast extract.....1.0g  
Resazurin.....1.0mg  
Salt base solution.....1.0L  
Na<sub>2</sub>S·9H<sub>2</sub>O solution.....10.0mL  
pH 7.2 ± 0.2 at 25°C

**Preparation of Sulfur:** Sterilize powdered elemental sulfur by steaming for 3 hr at 0 psi pressure–100°C on 3 successive days.

# **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

## **Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O.....0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

# **Salt Base Solution:**

## **Composition per liter:**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.....1.3g  
KH<sub>2</sub>PO<sub>4</sub>.....0.28g  
MgSO<sub>4</sub>·7H<sub>2</sub>O.....0.25g  
CaCl<sub>2</sub>·2H<sub>2</sub>O.....0.07g  
FeCl<sub>3</sub>·6H<sub>2</sub>O.....0.02g  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O.....4.5mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O.....1.8mg  
ZnSO<sub>4</sub>·7H<sub>2</sub>O.....0.22mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O.....0.05mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O.....0.03mg  
VOSO<sub>4</sub>.....0.03mg  
CoSO<sub>4</sub>·7H<sub>2</sub>O.....0.02mg

**Preparation of Salt Base Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to salt base solution and bring volume to 1.0L. Mix thoroughly. Adjust medium pH to 7.2 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Immediately prior to use, aseptically and anaerobically add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermococcus profundus*.

# ***Thermococcus stetteri* Medium**

## **Composition per liter:**

NaCl.....25.0g  
Sulfur.....10.0g  
Yeast extract.....3.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.....1.3g  
Peptone.....0.5g  
KH<sub>2</sub>PO<sub>4</sub>.....0.28g  
MgSO<sub>4</sub>·7H<sub>2</sub>O.....0.25g  
CaCl<sub>2</sub>·2H<sub>2</sub>O.....0.07g  
FeCl<sub>2</sub>·2H<sub>2</sub>O.....0.02g  
NaB<sub>4</sub>O·10H<sub>2</sub>O.....4.5mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O.....1.8mg  
Resazurin.....1.0mg  
ZnSO<sub>4</sub>·7H<sub>2</sub>O.....0.22mg

CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
NaMoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> .....	0.01mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
pH 6.5 ± 0.2 at 25°C	

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.5. Do not autoclave. Sterilize by steaming at 100°C for 30 min on 3 consecutive days. Before inoculation, add 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation of *Thermococcus stetteri*.

***Thermodesulfobacterium Medium*****Composition per liter:**

Na <sub>2</sub> SO <sub>4</sub> .....	3.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.5mg
Resazurin .....	1.0mg
Sodium lactate solution .....	20.0mL
Trace elements solution .....	10.0mL
Yeast extract solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	5.0mL

pH 6.8 ± 0.2 at 25°C

**Sodium Lactate Solution:****Composition per 20.0mL:**

Sodium lactate .....	4.0g
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**Preparation of Sodium Lactate Solution:** Add sodium lactate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition per liter:**

Nitritotriacetic acid .....	12.8g
NaCl .....	1.0g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.20
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.17g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.1g
ZnCl <sub>2</sub> .....	0.1g
CuCl <sub>2</sub> .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.026g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Yeast Extract Solution:****Composition per 10.0mL:**

Yeast extract .....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Prior to use, neutralize solution by dropwise addition of sterile 1N HCl.

**Vitamin Solution:****Composition per liter:**

Pyridoxine·HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine·HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except sodium lactate solution, yeast extract solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Anaerobically distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add to 1.0L of medium 20.0mL of sterile sodium lactate solution, 10.0mL of sterile yeast extract solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Thermodesulfobacterium commune*.

***Thermodesulfurhabdus Medium*****Composition per liter:**

NaCl .....	10.0g
Na <sub>2</sub> SO <sub>4</sub> .....	7.0g
Sodium acetate·3H <sub>2</sub> O .....	6.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	0.5mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL
NaHCO <sub>3</sub> solution .....	variable

pH 6.8 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

#### **Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.15g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:**

#### **Composition per 10.0mL:**

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 2.0g

**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### **Trace Elements Solution SL-10:**

#### **Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Anaerobically distribute 9.8mL volumes into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution and 0.1mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution to each tube. Aseptically and anaerobically add a sufficient volume of sterile NaHCO<sub>3</sub> solution to each tube to bring the pH to 6.8.

**Use:** For the cultivation of *Thermodesulfotobacterium norvegicus*.

### ***Thermodesulfotobacterium* Agar**

#### **Composition per liter:**

Na<sub>2</sub>SO<sub>4</sub> ..... 30.0g  
Agar ..... 20.0g  
Sodium lactate ..... 4.0g  
Yeast extract ..... 1.0g  
Mineral solution 2 ..... 50.0mL  
Na<sub>2</sub>CO<sub>3</sub> solution ..... 50.0mL  
Mineral solution 1 ..... 25.0mL  
Cysteine-sulfide reducing agent ..... 20.0mL  
Wolfe's mineral solution ..... 10.0mL  
Wolfe's vitamin solution ..... 10.0mL  
Resazurin (0.025% solution) ..... 4.0mL

pH 7.2 ± 0.2 at 25°C

### **Mineral Solution 1:**

#### **Composition per liter:**

K<sub>2</sub>HPO<sub>4</sub> ..... 6.0g

**Preparation of Mineral Solution 1:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Mineral Solution 2:**

#### **Composition per liter:**

NaCl ..... 12.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 6.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 6.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.4g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 1.6g

**Preparation of Mineral Solution 2:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Na<sub>2</sub>CO<sub>3</sub> Solution:**

#### **Composition per 100.0mL:**

Na<sub>2</sub>CO<sub>3</sub> ..... 8.0g

**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

### **Cysteine-Sulfide Reducing Agent:**

#### **Composition per 20.0mL:**

L-Cysteine·HCl·H<sub>2</sub>O ..... 300.0mg  
Na<sub>2</sub>S·9H<sub>2</sub>O ..... 300.0mg

### **Preparation of Cysteine-Sulfide Reducing Agent:**

Add L-cysteine·HCl·H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. In a separate tube, add Na<sub>2</sub>S·9H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. Gas both solutions with 100% N<sub>2</sub> and cap tubes. Autoclave both solutions for 15 min at 15 psi pressure–121°C using fast exhaust. Cool to 50°C. Aseptically combine the two solutions under 100% N<sub>2</sub>.

### **Wolfe's Mineral Solution:**

#### **Composition per liter**

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 3.0g  
Nitriloacetic acid ..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·H<sub>2</sub>O ..... 0.5g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub> ..... 0.1g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.01g  
AlK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O ..... 0.01g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitriloacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

### **Wolfe's Vitamin Solution:**

#### **Composition per liter:**

Pyridoxine·HCl ..... 10.0mg  
Thiamine·HCl ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
Calcium pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Thioctic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Cyanocobalamin ..... 100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution and cysteine-sulfide reducing agent, to dis-

tilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically add the sterile vitamin solution and then the sterile cysteine-sulfide reducing agent. Adjust the pH to 7.2. Distribute aseptically and anaerobically into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermodesulfovibrio commune* and other *Thermodesulfovibrio* species.

### *Thermodesulfovibrio yellowstonii* Medium (DSMZ Medium 749)

#### Composition per liter:

Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
Na-lactate.....	2.5g
NaHCO <sub>3</sub> .....	1.3g
KCl.....	0.5g
Yeast extract.....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4g
NH <sub>4</sub> Cl.....	0.25g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.2g
Na-thioglycolate.....	0.2g
L-Ascorbic acid.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.15g
Resazurin.....	0.5mg
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL

pH 7.5 ± 0.2 at 25°C

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 100% N<sub>2</sub>. Add components, except vitamin solution, Na-thioglycolate, NaHCO<sub>3</sub>, and L-ascorbic acid, to distilled/deionized water and bring volume to 990.0L. Mix thoroughly. Gently heat and bring to boiling. Cool while sparging with 100% N<sub>2</sub>. Add 0.2g Na-thioglycolate, 1.3g NaHCO<sub>3</sub>, and 0.2g L-ascorbic acid. Mix thoroughly. Adjust pH to 7.5. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL sterile vitamin solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thermodesulfovibrio yellowstonii*.

### *Thermofilum pendens* Medium

#### Composition per liter:

Sulfur, powdered.....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.07g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01mg
Yeast extract solution.....	20.0mL
Sucrose solution.....	20.0mL
Polar lipid fraction.....	6.0–12.0mL

pH 5.2 ± 0.2 at 25°C

#### Yeast Extract Solution:

##### Composition per 20.0mL:

Yeast extract.....	2.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for a few minutes. Sparge with 100% N<sub>2</sub>. Do not autoclave.

#### Sucrose Solution:

##### Composition per 20.0mL:

Sucrose.....	2.0g
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**Preparation of Sucrose Solution:** Add sucrose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% N<sub>2</sub>.

**Preparation of Sulfur:** Add 10.0g of powdered sulfur to a flask and sterilize by steaming for 3 hr on 3 consecutive days.

#### Polar Lipid Fraction:

##### Composition per 20.0mL:

<i>Thermoproteus tenax</i> cells (wet weight).....	10.0g
Chloroform.....	500.0mL
Acetone.....	500.0mL
Methanol.....	500.0mL
TA buffer solution.....	80.0mL
Chloroform/methanol 1:1 (v/v).....	20.0mL

#### TA Buffer Solution:

##### Composition per 100.0mL:

Tris-HCl.....	0.79g
β-mercaptoethanol.....	0.78g

NH <sub>4</sub> Cl.....	0.118g
EDTA.....	0.029g

**Preparation of TA Buffer Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Polar Lipid Fraction:** Add 10.0g (wet weight) of *Thermoproteus tenax* cells to 20.0mL of TA buffer solution. Mix thoroughly. Sonicate for 2 min. Centrifuge at 20,000 rpm for 20 min. Resuspend pellet in 20.0mL of fresh TA buffer solution. Recentrifuge at 20,000 rpm for 20 min. Again resuspend pellet in 20.0mL of fresh TA buffer solution. Recentrifuge at 20,000 rpm for 20 min. Resuspend pellet in 20.0mL of fresh TA buffer solution. Centrifuge at 5,000 rpm for 5 min. Decant the supernatant solution and discard the pellet. Extract the supernatant solution twice with 20.0mL of chloroform/methanol (1:1) each time. Chromatograph the extract on a SIL-LC (325 mesh) silicic acid column (20 cm × 2 cm) using 500.0mL of chloroform, followed by 500.0mL of acetone, followed by 500.0mL of methanol. The methanol fraction is further purified by DEAE chromatography using chloroform/methanol 1:1 and methanol.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except yeast extract solution, sucrose solution, sulfur, and polar lipid fraction, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Anaerobically distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add to 1.0L of medium, 20.0mL of sterile yeast extract solution, 20.0mL of sterile sucrose solution, 10.0g of sterile sulfur, and 6.0–12.0mL of sterile polar lipid fraction. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Thermofilum pendens*.

#### Thermoleophilum Medium

**Composition per liter:**

NaNO <sub>2</sub> .....	2.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
KCl.....	0.04g
NaH <sub>2</sub> PO <sub>4</sub> .....	90.0mg
CaCl <sub>2</sub> .....	15.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	70.0µg
H <sub>3</sub> BO <sub>3</sub> .....	10.0µg
MnSO <sub>4</sub> ·5H <sub>2</sub> O.....	10.0µg
MoO <sub>3</sub> .....	10.0µg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0µg
<i>n</i> -Heptadecane.....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except *n*-heptadecane, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mL of *n*-heptadecane. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thermoleophilum album* and *Thermoleophilum minutum*.

#### Thermomicrobium fosteri Agar

**Composition per liter:**

Agar.....	20.0g
NH <sub>4</sub> Cl.....	2.0g

Na <sub>2</sub> HPO <sub>4</sub> .....	0.21g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
NaH <sub>2</sub> PO <sub>4</sub> .....	0.09g
KCl.....	0.04g
CaCl <sub>2</sub> .....	0.015g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	70.0µg
H <sub>3</sub> BO <sub>3</sub> .....	10.0µg
MnSO <sub>4</sub> ·5H <sub>2</sub> O.....	10.0µg
MoO <sub>3</sub> .....	10.0µg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0µg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.0mg
Heptadecane, filter sterilized.....	20.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except heptadecane, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile heptadecane. Mix thoroughly. Aseptically distribute into sterile tubes. Cool tubes rapidly in a slanted position.

**Use:** For the cultivation and maintenance of *Thermomicrobium fosteri*.

#### Thermomicrobium roseum Agar (DSMZ Medium 592)

**Composition per liter:**

Agar.....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (sublimed).....	1.3g
Yeast extract.....	1.0g
Tryptone.....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.247g
KH <sub>2</sub> PO <sub>4</sub> .....	0.280g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.074g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.019g
Salt solution.....	1.0mL

pH 8.5 ± 0.2 at 25°C

**Salt Solution:**

**Composition per liter:**

Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	4.4g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.8g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22g
CuCl <sub>2</sub> ·H <sub>2</sub> O.....	0.05g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.03g
VOSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03g

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Adjust pH to 2.0. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.5. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Pour into Petri dishes or pour short slants with a long butt in screw-capped tubes.

**Use:** For the cultivation and maintenance of *Thermomicrobium roseum*.

#### Thermomonospora Medium

**Composition per liter:**

Sucrose.....	30.0g
Agar.....	15.0g
Casamino acids.....	6.0g
NaNO <sub>3</sub> .....	3.0g
Yeast extract.....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g



KCl.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01g
pH 8.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Thermomonospora alba* and *Thermomonospora mesophila*.

#### **Thermophilic *Bacillus* Medium**

**Composition** per liter:

Peptone.....	8.0g
Yeast extract.....	4.0g
NaCl.....	3.0g
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of a variety of thermophilic *Bacillus* species.

#### **Thermophilic Hydrogen-Bacteria Medium**

**Composition** per 1000.5mL:

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O.....	4.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NaCl.....	1.0g
NH <sub>4</sub> NO <sub>3</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	10.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
Trace elements solution.....	0.5mL
pH 7.0 ± 0.2 at 25°C	

**Trace Elements Solution:**

**Composition** per liter:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	28.0mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	4.0mg
H <sub>3</sub> BO <sub>3</sub> .....	4.0mg
MnSO <sub>4</sub> ·5H <sub>2</sub> O.....	4.0mg
MoO <sub>3</sub> .....	4.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	2.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Incubate cultures in 5% O<sub>2</sub> + 80% H<sub>2</sub> + 10% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Hydrogenobacter thermophilus* and *Pseudomonas* species.

#### **Thermophilic Maintenance Medium**

**Composition** per liter:

NaHCO <sub>3</sub> .....	3.0g
Yeast extract.....	1.0g
NH <sub>4</sub> Cl.....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
Cysteine-sulfide reducing solution.....	40.0mL
Fructose solution.....	25.0mL

Wolfe's vitamin solution.....	10.0mL
Wolfe's mineral solution.....	10.0mL
Resazurin (0.01% solution).....	1.0mL
pH 5.6 ± 0.2 at 25°C	

#### **Cysteine-Sulfide Reducing Solution:**

**Composition** per 100.0mL:

Cysteine·HCl·H <sub>2</sub> O.....	1.25g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	1.25g

**Preparation of L-Cysteine-Sulfide Reducing Solution:** Add L-cysteine·HCl·H<sub>2</sub>O and Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### **Fructose Solution:**

**Composition** per 100.0mL:

Fructose.....	20.0g
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**Preparation of Fructose Solution:** Add fructose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### **Wolfe's Vitamin Solution:**

**Composition** per liter:

Pyridoxine·HCl.....	0.01g
Thiamine·HCl.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
Calcium pantothenate.....	5.0mg
<i>p</i> -Aminobenzoic acid.....	5.0mg
Thioctic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Cyanocobalamin.....	100.0µg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Wolfe's Mineral Solution:**

**Composition** per liter

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitriloacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.1g
CaCl <sub>2</sub> .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except fructose solution, to distilled/deionized water and bring volume to 935.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling until resazurin turns colorless, indicating reduction. Add 40.0mL of the cysteine-sulfide reducing solution. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C under O<sub>2</sub>-free 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Add 25.0mL of the sterile fructose solution. Adjust the pH to 5.6 if necessary. Aseptically and anaerobically distribute into sterile tubes. Cap with rubber stoppers.

**Use:** For the cultivation and maintenance of a variety of thermophilic anaerobes, including *Clostridium thermoautotrophicum*.

### Thermophilic Methanosarcina Medium

**Composition** per 1021.0mL:

NaCl .....	2.25g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
NaHCO <sub>3</sub> .....	0.85g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NH <sub>4</sub> Cl .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.348g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg
Resazurin .....	1.0mg
Rumen fluid, clarified .....	50.0mL
Methanol solution .....	10.0mL
Vitamin solution .....	10.0mL
L-Cysteine-HCl·H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 6.5–6.8 at 25°C

### Methanol Solution:

**Composition** per 10.0mL:

Methanol .....	5.0mL
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**Preparation of Methanol Solution:** Add methanol to distilled/deionized water and bring volume to 10.0mL. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Calcium DL-pantothenate .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

### L-Cysteine-HCl·H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

L-Cysteine-HCl .....	0.3g
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**Preparation of L-Cysteine-HCl·H<sub>2</sub>O Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C.

### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
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CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

### Preparation of Trace Elements Solution SL-10:

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>.

**Preparation of Medium:** Add components, except methanol solution, L-cysteine-HCl·H<sub>2</sub>O solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Sparge under 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 3–4 min. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile vitamin solution, 10.0mL of sterile L-cysteine-HCl·H<sub>2</sub>O solution, and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile screw-capped bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Methanosarcina thermophila*.

### Thermophilic Methanothrix Medium

**Composition** per liter:

NH <sub>4</sub> Cl .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
Resazurin .....	1.0mg
NaHCO <sub>3</sub> solution .....	20.0mL
Trace elements .....	10.0mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O solution .....	10.0mL
Sodium acetate solution .....	10.0mL
Vitamin solution .....	10.0mL
Coenzyme M solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	5.0mL

pH 6.5 ± 0.2 at 25°C

### NaHCO<sub>3</sub> Solution:

**Composition** per 20.0mL:

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 15 min. Autoclave for 15 min at 15 psi pressure–121°C.

### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrotriactic acid to approximately 500.0mL of distilled/deionized water. Dissolve by adding KOH and adjust pH to 6.5. Add remaining components. Bring volume to 1.0L with additional distilled/deionized water. Adjust pH to 7.0 with KOH.

**CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g

**Preparation of CaCl<sub>2</sub>·2H<sub>2</sub>O Solution:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Sodium Acetate Solution:**

**Composition per 10.0mL:**

Sodium acetate ..... 3.3g

**Preparation of Sodium Acetate Solution:** Add sodium acetate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:**

**Composition per liter:**

Pyridoxine-HCl ..... 10.0mg  
Calcium DL-pantothenate ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Riboflavin ..... 5.0mg  
Thiamine-HCl ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Coenzyme M Solution:**

**Composition per 10.0mL:**

Coenzyme M ..... 0.142g

**Preparation of Coenzyme M Solution:** Add coenzyme M to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10.0mL:**

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, CaCl<sub>2</sub>·2H<sub>2</sub>O solution, sodium acetate solution, vitamin solution, Coenzyme M solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 935.0mL. Gently heat and bring to boiling. Continue boiling for 10 min. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Gas the medium until pH reaches 5.8. Anaerobically distribute the medium into serum bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 20.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile CaCl<sub>2</sub>·2H<sub>2</sub>O solution, 10.0mL of sterile sodium acetate solution, 10.0mL of sterile vitamin solution, 10.0mL of sterile Coenzyme M solution,

and 5.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Bring gas atmosphere in each bottle to 30% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Methanothrix thermophila*.

**Thermophilic Spirochete Medium**

**Composition per 1012.0mL:**

Solution A ..... 920.0mL  
Solution D ..... 50.0mL  
Solution E (Vitamin solution) ..... 20.0mL  
Solution F ..... 10.0mL  
Solution G ..... 10.0mL  
Solution B (Trace elements solution SL-10) ..... 1.0mL  
Solution C (Selenite-tungstate solution) ..... 1.0mL  
pH 6.9 ± 0.2 at 25°C

**Solution A:**

**Composition per 920.0mL:**

NaCl ..... 4.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.8g  
KCl ..... 0.5g  
NH<sub>4</sub>Cl ..... 0.3g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.2g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.03g  
Resazurin ..... 1.0mg

**Preparation of Solution A:** Prepare and dispense under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 920.0mL. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for a few minutes. Cool to room temperature while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Anaerobically distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution B (Trace Elements Solution SL-10):**

**Composition per liter:**

FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 190.0mg  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 100.0mg  
ZnCl<sub>2</sub> ..... 70.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 36.0mg  
NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
H<sub>3</sub>BO<sub>3</sub> ..... 6.0mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg  
HCl (25% solution) ..... 10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution C (Selenite-Tungstate Solution):**

**Composition per liter:**

NaOH ..... 0.5g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg  
Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O ..... 4.0mg

**Preparation of Solution C (Selenite-Tungstate Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution D:**

**Composition per 50.0mL:**

NaHCO<sub>3</sub> ..... 3.0mg

**Preparation of Solution D:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Solution E (Vitamin Solution):

## Composition per liter:

Pyridoxine-HCl	10.0mg
Calcium DL-pantothenate	5.0mg
Lipoic acid	5.0mg
Nicotinic acid	5.0mg
p-Aminobenzoic acid	5.0mg
Riboflavin	5.0mg
Thiamine-HCl	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Solution F:

## Composition per 10.0mL:

Starch, soluble	1.0g
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**Preparation of Solution F:** Add starch to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

# Solution G:

## Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.3g
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**Preparation of Solution G:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. To 920.0mL of sterile solution A, aseptically and anaerobically add 1.0mL of sterile solution B, 1.0mL of sterile solution C, 50.0mL of sterile solution D, 20.0mL of sterile solution E, 10.0mL of sterile solution F, and 10.0mL of sterile solution G in that order. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Spirochaeta thermophila*.

## Thermophilic Streptomycete Medium

### Composition per liter:

Agar	20.0g
Maltose	20.0g
Soybean meal	5.0g
Yeast extract	2.0g

pH 6.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of thermophilic streptomycetes.

## Thermophilic Streptomycete Medium Ia

### Composition per liter:

Agar	20.0g
Sucrose	5.0g
Pancreatic digest of casein	5.0g
Yeast extract	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g
Dung extract	5.0mL

Molasses	5.0mL
Trace elements solution	1.0mL

pH 7.2 ± 0.2 at 25°C

## Dung Extract:

### Composition per 100.0mL:

Sheep manure, dried	25.0g
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**Preparation of Dung Extract:** Add dried sheep manure to 100.0mL of tap water. Mix thoroughly. Autoclave for 30 min at 15 psi pressure–121°C. Filter through Whatman #1 filter paper. Store at 4°C under toluene.

## Trace Elements Solution:

### Composition per 100.0mL:

Fe(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.1g
ZnSO <sub>4</sub>	0.1g
MnSO <sub>4</sub>	0.05g
CoSO <sub>4</sub>	0.01g
H <sub>3</sub> BO <sub>3</sub>	0.01g
CuSO <sub>4</sub>	8.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of thermophilic streptomycetes.

## Thermoplasma acidophilum Growth Medium 7B

### Composition per liter:

Sucrose	17.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	6.8g
KOH	1.22g
Yeast extract	1.0g
MgSO <sub>4</sub>	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.25g
H <sub>3</sub> PO <sub>4</sub> solution	1.5mL
Antifoam A	10.0μL

pH 1.60 ± 0.2 at 25°C

## H<sub>3</sub>PO<sub>4</sub> Solution:

### Composition per 100.0mL:

H <sub>3</sub> PO <sub>4</sub>	85.0g
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**Preparation of H<sub>3</sub>PO<sub>4</sub> Solution:** Add H<sub>3</sub>PO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Preparation of Medium:** Add components, except Antifoam A, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 1.60 with 50% H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Sterilize by heating to 100°C for 30 min. Allow to stand at room temperature for 24 hr. Add 10.0μL of Antifoam A per liter.

**Use:** For the cultivation of *Thermococcus acidophilum*.

## Thermoplasma acidophilum Medium

### Composition per liter:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.32g
Yeast extract solution	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.372g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.247g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.074g
Glucose solution	20.0mL

Yeast extract solution .....	10.0mL
Trace elements solution .....	10.0mL
pH 1.0–2.0 at 25°C	

**Glucose Solution:****Composition** per 20.0mL:

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Yeast Extract Solution:****Composition** per 10.0mL:

Yeast extract .....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Trace Elements Solution:****Composition** per liter:

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	1.93g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	0.45g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	22.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg
VOSO <sub>4</sub> ·5H <sub>2</sub> O .....	3.8mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	3.0mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except glucose solution and yeast extract solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 1.0–2.0 with 10N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 20.0mL of sterile glucose solution and 10.0mL of sterile yeast extract solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thermoplasma acidophilum*.

***Thermoplasma acidophilum* Medium 7A****Composition** per liter:

Glucose .....	10.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	6.8g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
pH 1.65 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 1.65 with 50% H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Sterilize by heating to 100°C for 30 min.

**Use:** For the cultivation of *Thermococcus acidophilum*.

***Thermoplasma* Agar****Composition** per liter:

Basal solution .....	450.0mL
Solution B .....	450.0mL
Solution C .....	100.0mL
pH 2.0 ± 0.2 at 25°C	

**Basal Solution:****Composition** per 500.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g

**Preparation of Basal Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 2.0 with 10N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C.

**Solution B:****Composition** per 450.0mL:

Noble agar .....	12.0g
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**Preparation of Solution B:** Add agar to distilled/deionized water and bring volume to 450.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C.

**Solution C:****Composition** per 100.0mL:

Glucose .....	10.0g
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**Preparation of Solution C:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine the cooled, sterile basal medium with sterile solution B and sterile solution C. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermoplasma acidophilum* and other *Thermoplasma* species.

***Thermoplasma* Broth****Composition** per liter:

Basal solution .....	500.0mL
Solution C .....	100.0mL
pH 2.0 ± 0.2 at 25°C	

**Basal Solution:****Composition** per 500.0mL:

KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
Yeast extract .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g

**Preparation of Basal Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 2.0 with 10N H<sub>2</sub>SO<sub>4</sub>.

**Solution C:****Composition** per 100.0mL:

Glucose .....	10.0g
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**Preparation of Solution C:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add 500.0mL of basal solution to 400.0mL of distilled/deionized water. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 55°C. Aseptically add 100.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermoplasma acidophilum* and other *Thermoplasma* species.

### ***Thermoplasma volcanium* Medium**

#### **Composition per liter:**

KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
Glucose solution .....	10.0mL
Yeast extract solution.....	10.0mL

pH 6.5 ± 0.2 at 25°C

#### **Glucose Solution:**

##### **Composition per 10.0mL:**

Glucose .....	5.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

#### **Yeast Extract Solution:**

##### **Composition per 10.0mL:**

Yeast extract.....	1.0g
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**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution and yeast extract solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Adjust pH to 2.0 with 10N H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile glucose solution and 10.0mL of sterile yeast extract solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks. Final pH should be 2.0–3.0.

**Use:** For the aerobic cultivation and maintenance of *Thermoplasma volcanium*.

### ***Thermoproteus* Medium**

#### **Composition per liter:**

Solution A .....	500.0mL
Solution B .....	450.0mL
Solution C .....	50.0mL

pH 4.8–5.6 at 25°C

#### **Solution A:**

##### **Composition per 500.0mL:**

Glucose .....	10.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.556g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.492g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.344g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.264g
Yeast extract.....	0.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.014g
Resazurin .....	1.0mg
Trace elements .....	10.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Immediately filter sterilize.

#### **Trace Elements:**

##### **Composition per liter:**

Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O.....	0.45g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.022g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	5.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	3.6mg
VOSO <sub>4</sub> ·5H <sub>2</sub> O .....	3.6mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.2mg

**Preparation of Trace Elements:** Add components to distilled/deionized water and bring volume to 1.0L. Mix

thoroughly. Adjust pH to 3.0 with H<sub>2</sub>SO<sub>4</sub> to retard precipitation.

#### **Solution B:**

##### **Composition per 450.0mL:**

Sulfur .....	10.0g
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**Preparation of Solution B:** Add sulfur to 450.0mL of distilled/deionized water. Autoclave for 30 min at 0 psi pressure–100°C on 3 consecutive days.

#### **Solution C:**

##### **Composition per 50.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.85g
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**Preparation of Solution C:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine solutions A, B, and C under 97% N<sub>2</sub> + 3% H<sub>2</sub>. Adjust pH to 4.8–5.6 with H<sub>2</sub>SO<sub>4</sub>. Aseptically and anaerobically distribute into sterile tubes or flasks under 97% N<sub>2</sub> + 3% H<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Thermoproteus tenax* and other *Thermoproteus* species.

### ***Thermoproteus neutrophilus* Medium**

#### **Composition per liter:**

Sulfur, powdered .....	8.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
NaHCO <sub>3</sub> .....	0.85g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
Na <sub>2</sub> B <sub>4</sub> ·10H <sub>2</sub> O.....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8mg
Resazurin .....	0.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.03mg
VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> .....	0.01mg
Dithionite solution.....	1.0mL

pH 6.5 ± 0.2 at 25°C

#### **Dithionite Solution:**

##### **Composition per 10.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> .....	0.25g
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**Preparation of Dithionite Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except sulfur, NaHCO<sub>3</sub>, resazurin, and dithionite solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add sulfur and resazurin. Adjust pH to 6.5 with NaOH. Gently heat and bring to boiling. Continue boiling for 5 min. Cool to room temperature while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add NaHCO<sub>3</sub>. Mix thoroughly. Continue to sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub> until pH reaches 6.5. Distribute anaerobically 10.0mL of medium into 30.0mL serum bottles. Sterilize medium by heating at 85°C for 1 hr on 3 consecutive days. Prior to inoculation, add 10.0μL of sterile dithionite solution to each bottle.

**Use:** For the aerobic cultivation and maintenance of *Thermoproteus neutrophilus*.

***Thermosipho africanus* Medium****Composition per liter:**

NaHCO <sub>3</sub> .....	4.0g
Sodium acetate.....	4.0g
Sodium formate.....	2.0g
Yeast extract.....	1.0g
L-Cysteine-HCl.....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4g
NaCl.....	0.4g
NH <sub>4</sub> Cl.....	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.05g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2.0mg
Resazurin.....	1.0mg
Sludge fluid.....	50.0mL
Fatty acid mixture.....	20.0mL
Trace elements solution SL-10.....	1.0mL
pH 6.7 ± 0.2 at 25°C	

**Sludge Fluid:****Composition per 100.0mL:**

Sludge.....	100.0mL
Yeast extract.....	0.4g

**Preparation of Sludge Fluid:** To 100.0mL of sludge from an anaerobic digester, add 0.4g of yeast extract. Sparge with 100% N<sub>2</sub> for a few minutes. Incubate at 37°C for 24 hr. Centrifuge the sludge at 13,000 × g for 15 min. Decant the clear supernatant solution. Sparge with 100% N<sub>2</sub> for a few minutes. Store in screw-capped bottles at room temperature in the dark.

**Fatty Acid Mixture:****Composition per 20.0mL:**

α-Methylbutyric acid.....	0.5g
Isobutyric acid.....	0.5g
Isovaleric acid.....	0.5g
Valeric acid.....	0.5g

**Preparation of Fatty Acid Mixture:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Adjust pH to 7.5 with concentrated NaOH.

**Trace Elements Solution SL-10:****Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thermosipho africanus*.

***Thermosphaera* Medium  
(DSMZ Medium 817)****Composition per liter:**

MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.2g
Yeast extract.....	1.0g
Peptone.....	1.0g
NaCl.....	0.9g
KCl.....	17.0mg
NH <sub>4</sub> Cl.....	12.5mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	7.0mg
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O.....	7.0mg
Resazurin.....	0.4mg
FeCl <sub>3</sub> .....	0.05mg
Vitamin solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
NaHCO <sub>3</sub> solution.....	10.0mL

pH 6.5 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition per 10.0mL:**

NaHCO <sub>3</sub> .....	1.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, vitamin solution, and NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Flush medium with 80% N<sub>2</sub> + 20% CO<sub>2</sub> for 5 min. Adjust medium pH to 6.53. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL sterile vitamin solution, and 10.0mL sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile thick walled tubes or thick walled bottles. Pressurize with 2 bar atmosphere of 80% N<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation of *Thermosphaera aggregans*.

### Thermosyntropha Medium (DSMZ Medium 731)

#### Composition per liter:

Yeast extract	10.0g
Na <sub>2</sub> CO <sub>3</sub>	3.0g
NH <sub>4</sub> Cl	1.0g
NaCl	0.5g
K <sub>2</sub> HPO <sub>4</sub>	0.3g
KCl	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Resazurin	0.5mg
Vitamin solution	40.0mL
Trace elements solution	10.0mL
Cysteine solution	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution	10.0mL
NaHCO <sub>3</sub> solution	10.0mL
Selenite-tungstate solution	1.0mL

pH 8.5 ± 0.2 at 25°C

#### Selenite-Tungstate Solution:

##### Composition per liter:

NaOH	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### NaHCO<sub>3</sub> Solution:

##### Composition per 10.0mL:

NaHCO <sub>3</sub>	3.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

#### Cysteine Solution:

##### Composition per 10.0mL:

L-Cysteine-HCl·H <sub>2</sub> O	0.15g
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**Preparation of Cysteine Solution:** Add L-cysteine-HCl·H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.0g
Nitrilotriacetic acid	1.5g
NaCl	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	0.02g
H <sub>3</sub> BO <sub>3</sub>	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Pyridoxine-HCl	10.0mg
Thiamine-HCl·2H <sub>2</sub> O	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
D-Ca-pantothenate	5.0mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Biotin	2.0mg
Folic acid	2.0mg
Vitamin B <sub>12</sub>	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, cysteine solution, vitamin solution, selenite-tungstate solution, and trace elements solution, to distilled/deionized water and bring volume to 919.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Adjust pH to 8.5 with HCl. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL NaHCO<sub>3</sub> solution, 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL cysteine solution, 40.0mL vitamin solution, 1.0mL selenite-tungstate solution, and 10.0mL trace elements solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermosyntropha lipolytica*.

### Thermoterrabacterium Medium (DSMZ Medium 778)

#### Composition per liter:

NaHCO <sub>3</sub>	10.0g
Na <sub>2</sub> -9,10-anthraquinone-2,6-disulfonate	8.25g
Yeast extract	1.0g
KH <sub>2</sub> PO <sub>4</sub>	0.33g
NH <sub>4</sub> Cl	0.33g
KCl	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.33g
NiCl <sub>2</sub> ·6H <sub>2</sub> O	200.0μg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O	120.0μg
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O	30.0μg
Calcium chloride solution	10.0mL
Vitamin solution	10.0mL
Glycerol (87%)	3.0mL
Trace elements solution SL-10	1.0mL

pH 6.8 ± 0.2 at 25°C

#### Calcium Chloride Solution:

##### Composition per 10mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.33g
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**Preparation of Calcium Chloride:** Add CaCl<sub>2</sub>·2H<sub>2</sub>O to 10.0mL of distilled/deionized water. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.



**Trace Elements Solution SL-10:****Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin Solution:****Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub>, calcium chloride solution, and vitamin solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for several minutes to dissolve the anthraquinone. Cool to room temperature under atmosphere of 100% CO<sub>2</sub>. Add solid NaHCO<sub>3</sub>. Adjust pH to 6.8 with NaOH. Dispense medium under CO<sub>2</sub> into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically and anaerobically distribute into sterile tubes or bottles. Before use, add CaCl<sub>2</sub> and vitamins from anaerobic, sterile stock solution.

**Use:** For the cultivation of *Thermoterrabacterium ferrireducens*.

***Thermotoga elfii* Medium****Composition** per liter:

NaCl .....	10.0g
Pancreatic digest of casein .....	2.0g
Yeast extract .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KCl .....	0.1g
L-Cysteine-HCl·H <sub>2</sub> O .....	0.5g
Sodium acetate .....	0.5g
Resazurin .....	1.0mg
Na <sub>2</sub> CO <sub>3</sub> solution .....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	20.0mL
Wolfe's mineral solution .....	10.0mL

pH 8.0 ± 0.2 at 25°C

**NaHCO<sub>3</sub> Solution:****Composition** per 20.0mL:

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 20.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.4g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Before use, neutralize to pH 7.0 with sterile HCl.

**Modified Wolfe's Mineral Solution:****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.01g
NaWO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Modified Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Na<sub>2</sub>CO<sub>3</sub> solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 960.0mL. Mix thoroughly. Adjust pH to 8.0 with 10M KOH. Gently heat and bring to boiling. Cool to room temperature while sparging with 100% N<sub>2</sub>. Anaerobically dispense into tubes in 5.0mL aliquots under an atmosphere of 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 45 min at 6 psi pressure–110°C. Just prior to use, aseptically and anaerobically add 0.1mL of sterile Na<sub>2</sub>CO<sub>3</sub> solution and 0.1mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to each tube.

**Use:** For the cultivation of *Thermotoga elfii*.

***Thermotoga hypogea* Medium  
(DSMZ Medium 794)****Composition** per liter:

NaCl .....	10.0g
Yeast extract .....	2.0g
Trypticase .....	2.0g
NH <sub>4</sub> Cl .....	1.0g
Na-Acetate .....	0.5g
L-Cysteine .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> .....	0.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KCl .....	0.1g
Resazurin .....	0.5mg

Na-thiosulfate solution.....	20.0mL
NaHCO <sub>3</sub> solution.....	20.0mL
Xylose solution.....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	14.0mL
Trace elements solution.....	10.0mL
pH 7.3 ± 0.2 at 25°C	

**Xylose Solution:****Composition per 20.0mL:**

Xylose.....	3.0g
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**Preparation of Xylose Solution:** Add xylose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	2.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Must be prepared freshly.

**Na-thiosulfate Solution:****Composition per 20.0mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	5.0g
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**Preparation of Na-thiosulfate Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 20.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.6g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 20.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except xylose solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na-thiosulfate solution, and NaHCO<sub>3</sub> solution, to 926.0mL distilled/deionized water. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 7.2–7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Aseptically and anaerobically add 20.0mL sterile xylose solution, 14.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution, 20.0mL

Na-thiosulfate solution, and 20.0mL NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thermotoga hypogaea*.

**Thermotoga Medium****Composition per 1017.0mL:**

NaCl.....	20.0g
Starch, soluble.....	5.0g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
Yeast extract.....	0.5g
Resazurin.....	1.0mg
Artificial seawater.....	250.0mL
Trace elements solution.....	15.0mL

pH 6.5 ± 0.2 at 25°C

**Artificial Seawater:****Composition per liter:**

NaCl.....	27.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	7.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.25g
KCl.....	0.65g
NaBr.....	0.1g
H <sub>3</sub> BO <sub>3</sub> .....	30.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O.....	15.0mg
Citric acid.....	10.0mg
KI.....	0.05mg

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thermotoga maritima* and *Thermotoga neapolitana*.

**Thermotoga 2 Medium****Composition per 1015.0mL:**

Starch, soluble.....	5.0g
NaCl.....	3.46g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.88g

EDTA·Na <sub>2</sub> .....	0.768g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.69g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5g
Yeast extract.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
KCl.....	0.08g
NaBr.....	12.5mg
H <sub>3</sub> BO <sub>3</sub> .....	3.75mg
(NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> ) <sub>2</sub> .....	3.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O.....	1.9mg
Resazurin.....	1.0mg
KI.....	0.006mg
Trace elements solution.....	15.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thermotoga thermarum*.

***Thermotoga petrophila* Medium  
(DSMZ Medium 913)**

**Composition per liter:**

MOPS.....	5.0g
Yeast extract.....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Resazurin.....	0.5mg
Artificial seawater.....	750.0mL
Trace elements solution.....	10.0mL
Vitamin solution.....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Artificial Seawater:****Composition per liter:**

NaCl.....	27.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	7.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.5g
KCl.....	0.65g
NaBr.....	0.1g
H <sub>3</sub> BO <sub>3</sub> .....	30.0mg
SrCl <sub>2</sub> ·6H <sub>2</sub> O.....	15.0mg
Citric acid.....	10.0mg

KI.....	0.05mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.25 g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition per 10.0mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Add components, except vitamin solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to 750.0mL artificial seawater. Bring volume to 980.0mL with distilled/deionized water. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Distribute under 100% N<sub>2</sub> into anaerobe tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium, 10.0mL vitamin solution and 10.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. The final pH of the medium should be 7.0.

**Use:** For the cultivation of *Thermotoga petrophila* and *Thermotoga naphthophila*.

***Thermotoga subterranea* Medium****Composition per liter:**

NaCl .....	12.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
PIPES .....	3.4g
KCl .....	2.0g
Peptone .....	1.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
Yeast extract .....	0.5g
NH <sub>4</sub> Cl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.025g
K <sub>2</sub> HPO <sub>4</sub> .....	0.02g
Resazurin .....	0.5mg
Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Wolfe's Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Wolfe's Mineral Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except Wolfe's vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL of sterile Wolfe's vitamin solution. Mix thoroughly. Adjust medium pH to 7.5 by adding sterile anaerobic 1N NaOH. Aseptically and anaerobically distribute into sterile tubes or bottles.

**Use:** For the cultivation of *Thermotoga subterranea*.

***Thermovenabulum* Medium  
(DSMZ Medium 962)****Composition per liter:**

NaHCO <sub>3</sub> .....	0.7g
NH <sub>4</sub> Cl .....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.33g
KCl .....	0.33g
Yeast extract .....	0.05g
Meat extract solution .....	50.0mL
Sodium fumarate solution .....	50.0mL
Vitamin solution .....	10.0mL
Trace elements solution .....	10.0mL
Selenite-tungstate solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

**Selenite-Tungstate Solution:****Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Vitamin Solution:****Composition per liter:**

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
<i>p</i> -Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

**Sodium Fumarate Solution:****Composition per 50.0mL:**

Na <sub>2</sub> -fumarate .....	3.20g
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**Preparation of Sodium Fumarate Solution:** Add Na<sub>2</sub>-fumarate to distilled/deionized water and bring volume to 50.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Meat Extract Solution:**

**Composition per 50.0mL:**

Meat extract ..... 3.0g

**Preparation of Meat Extract Solution:** Add meat extract to distilled/deionized water and bring volume to 50.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub>, vitamin solution, sodium fumarate solution, and meat extract solution, to distilled/deionized water and bring volume to 890.0mL. Mix thoroughly. Gently heat and bring to boiling. Boil for 3 min. Cool to 25°C while sparging with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Add solid NaHCO<sub>3</sub>. Adjust pH to 6.8–7.0. Distribute into anaerobe tubes or bottles under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per liter of medium, 10.0mL sterile vitamin solution, 50.0mL sterile meat extract solution, and 50.0mL sterile sodium fumarate solution. Mix thoroughly. The final pH should be 6.5.

**Use:** For the cultivation of *Thermovibrio ferriorganovorum*.

***Thermovibrio Medium*  
(DSMZ Medium 961)**

**Composition per liter:**

Synthetic seawater ..... 970.0mL  
Potassium nitrate solution ..... 10.0mL  
Yeast extract solution ..... 10.0mL  
Na<sub>2</sub>S-9H<sub>2</sub>O solution ..... 10.0mL  
pH 6.5 ± 0.2 at 25°C

**Synthetic Seawater:**

**Composition per liter:**

NaCl ..... 27.7g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 7.0g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 5.5g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.75g  
KCl ..... 0.65g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.5g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.25g  
NaBr ..... 0.1g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.03g  
SrCl<sub>2</sub>·6H<sub>2</sub>O ..... 15.0mg  
Resazurin ..... 1.0mg  
KI ..... 0.05mg

**Preparation of Synthetic Seawater:** Add components to distilled water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>S-9H<sub>2</sub>O Solution:**

**Composition per 10mL:**

Na<sub>2</sub>S-9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S-9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S-9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Yeast Extract Solution:**

**Composition per 10.0mL:**

Yeast extract ..... 1.0g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to

10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Potassium Nitrate Solution:**

**Composition per 10.0mL:**

KNO<sub>3</sub> ..... 0.33g

**Preparation of Potassium Nitrate Solution:** Add KNO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of Medium:** Gently heat and bring 1.0L synthetic seawater to boiling. Boil for 3 min. Cool to 25°C while sparging with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 6.8–7.0. Distribute 20.0mL aliquots into 100mL serum bottles under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add per 20.0mL of medium, 0.2mL sterile potassium nitrate solution, 0.2mL sterile yeast extract solution, and 0.2mL sterile Na<sub>2</sub>S-9H<sub>2</sub>O solution. Mix thoroughly. Adjusted pH should be 6.5.

**Use:** For the cultivation of *Thermovibrio ruber*.

***Thermus Agar***

**Composition per liter:**

Agar ..... 28.0g  
Pancreatic digest of casein ..... 2.5g  
Yeast extract ..... 2.5g  
Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O ..... 0.43g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
Nitrilotriacetic acid ..... 0.1g  
KH<sub>2</sub>PO<sub>4</sub> ..... 54.0mg  
CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 40.0mg  
Micronutrient solution ..... 1.0mL  
Fe-citrate solution ..... 0.5mL  
pH 7.2 ± 0.2 at 25°C

**Micronutrient Solution:**

**Composition per liter:**

MnSO<sub>4</sub>·H<sub>2</sub>O ..... 2.28g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.5g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 45.0mg  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 25.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 25.0mg  
Concentrated H<sub>2</sub>SO<sub>4</sub> ..... 0.5mL

**Preparation of Micronutrient Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Fe-Citrate Solution:**

**Composition per liter:**

Fe-citrate ..... 24.5mg

**Preparation of Fe-Citrate Solution:** Add Fe-citrate to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add nitrilotriacetic acid to 100.0mL of distilled/deionized water. Mix thoroughly. Adjust pH to 6.5 with KOH. Add remaining components and bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermus* species.

### ***Thermus* BP Medium**

#### **(*Thermus* Beef Extract Polypeptone™ Medium)**

##### **Composition per liter:**

Agar .....	25.0g
Beef extract .....	4.0g
Polypeptone .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	3.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermus aquaticus* and other *Thermus* species.

### ***Thermus brockii* Medium**

##### **Composition per liter:**

Pancreatic digest of casein .....	1.0g
Yeast extract .....	1.0g
Salts solution .....	100.0mL

pH 7.6 ± 0.2 at 25°C

##### **Salts Solution:**

##### **Composition per liter:**

NaNO <sub>3</sub> .....	6.89g
KNO <sub>3</sub> .....	1.03g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
Nitritotriacetic acid .....	1.0g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.6g
NaCl .....	80.0mg
FeCl <sub>3</sub> solution .....	10.0mL
Trace elements solution .....	10.0mL

**Preparation of Salts Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 8.2 with 1M NaOH. Add remaining components. Add distilled/deionized water to 1.0L.

##### **FeCl<sub>3</sub> Solution:**

##### **Composition per 100.0mL:**

FeCl <sub>3</sub> .....	28.0mg
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**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

##### **Trace Elements Solution:**

##### **Composition per liter:**

MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.2g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	46.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	25.0mg
CuSO <sub>4</sub> .....	16.0mg
H <sub>2</sub> SO <sub>4</sub> .....	0.5mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thermus brockii*.

### ***Thermus* Enhanced Agar**

##### **Composition per liter:**

Agar .....	28.0g
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Pancreatic digest of casein .....	2.5g
Yeast extract .....	2.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Nitritotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg
Phosphate buffer solution .....	100.0mL
Ferric citrate solution .....	0.5mL
Trace elements solution .....	0.5mL

##### **Phosphate Buffer Solution:**

##### **Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	43.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.44g

**Preparation of Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

##### **Ferric Citrate Solution:**

##### **Composition per 10.0mL:**

Ferric citrate .....	24.5mg
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

##### **Trace Elements Solution:**

##### **Composition per liter:**

Nitritotriacetic acid .....	12.8g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.3g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	50.0mg
H <sub>3</sub> BO <sub>3</sub> .....	20.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg

**Preparation of Trace Elements Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except phosphate buffer solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile phosphate buffer solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thermus* species.

### ***Thermus* Enhanced Agar with 1% NaCl**

##### **Composition per liter:**

Agar .....	28.0g
NaCl .....	10.0g
Pancreatic digest of casein .....	2.5g
Yeast extract .....	2.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Nitritotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg
Phosphate buffer solution .....	100.0mL
Ferric citrate solution .....	0.5mL
Trace elements solution .....	0.5mL

##### **Phosphate Buffer Solution:**

##### **Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	43.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.44g

**Preparation of Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50–55°C.

#### **Ferric Citrate Solution:**

##### **Composition** per 10.0mL:

Ferric citrate ..... 24.5mg

**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

#### **Trace Elements Solution:**

##### **Composition** per liter:

Nitrilotriacetic acid ..... 12.8g  
 FeCl<sub>2</sub>·4H<sub>2</sub>O ..... 1.0g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.5g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.3g  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 50.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 50.0mg  
 H<sub>3</sub>BO<sub>3</sub> ..... 20.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 20.0mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except phosphate buffer solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile phosphate buffer solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thermus* species.

#### ***Thermus* Medium**

##### **Composition** per liter:

Agar ..... 30.0g  
 Polypeptone ..... 8.0g  
 Yeast extract ..... 4.0g  
 NaCl ..... 2.0g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermus aquaticus* and other *Thermus* species.

#### ***Thermus* 162 Medium (DSMZ Medium 878)**

##### **Composition** per liter:

Agar ..... 28.0g  
 Yeast extract ..... 1.0g  
 Tryptone ..... 1.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 200.0mg  
 Nitrilotriacetic acid ..... 100.0mg  
 CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 40.0mg  
 Phosphate buffer ..... 100.0mL  
 Ferric citrate solution ..... 0.5mL  
 Trace elements solution ..... 0.5mL

pH 7.2 ± 0.2 at 25°C

#### **Ferric Citrate Solution:**

##### **Composition** per 10.0mL:

Ferric citrate ..... 24.5mg

**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

#### **Trace Elements Solution:**

CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 45.0g  
 CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 25.0g  
 Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O ..... 25.0g  
 MnSO<sub>4</sub>·2H<sub>2</sub>O ..... 2.28g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.5g  
 H<sub>2</sub>SO<sub>4</sub> ..... 0.5mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### **Phosphate Buffer:**

##### **Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O ..... 43.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 5.44g

**Preparation of Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Preparation of Medium:** Add components, except phosphate buffer, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Add 100.0mL warm phosphate buffer. Mix thoroughly. Pour into Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermus* spp., *Rubrobacter xylanophilus*, *Thermonema rossianum*, *Deinococcus geothermalis*, *Deinococcus murrayi*, and *Tepidimonas ignava*.

#### ***Thermus* 162 Medium**

##### **Composition** per 1010.0mL:

Agar ..... 28.0g  
 Tryptone ..... 2.5g  
 Yeast extract ..... 2.5g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2g  
 Nitrilotriacetic acid ..... 0.1g  
 CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 40.0mg  
 Phosphate buffer solution ..... 100.0mL  
 Ferric citrate solution ..... 0.5mL  
 Trace elements solution ..... 0.5mL

pH 7.2 ± 0.2 at 25°C

#### **Phosphate Buffer Solution:**

##### **Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O ..... 43.0g  
 KH<sub>2</sub>PO<sub>4</sub> ..... 5.44g

**Preparation of Phosphate Buffer Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Ferric Citrate Solution:**

##### **Composition** per 10.0mL:

Ferric citrate ..... 24.5mg

**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly.

# **Trace Elements Solution:**

## **Composition per liter:**

Nitrilotriacetic acid .....	12.8g
FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.3g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	50.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	50.0mg
H <sub>3</sub> BO <sub>3</sub> .....	20.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	20.0mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 7.0.

**Preparation of Medium:** Add components, except phosphate buffer solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 100.0mL of sterile phosphate buffer solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thermus* species.

## ***Thermus* Medium Enhanced**

### **Composition per liter:**

Agar .....	28.0g
Yeast extract .....	2.5g
Tryptone .....	2.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
Phosphate buffer solution .....	100.0mL
Ferric citrate .....	0.5mL
Trace elements solution .....	0.5mL

### **Ferric Citrate Solution:**

#### **Composition per 100.0mL:**

Ferric citrate .....	0.24g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

### **Phosphate Buffer Solution:**

#### **Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	43.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.44g

**Preparation of Phosphate Buffer Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C.

### **Trace Elements Solution:**

#### **Composition per liter:**

Nitrilotriacetic acid .....	12.8g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	1.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.3g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05g
H <sub>3</sub> BO <sub>3</sub> .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except phosphate buffer solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile phosphate buffer solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thermus* species.

## ***Thermus* Medium Enhanced with 1% NaCl**

### **Composition per liter:**

Agar .....	28.0g
NaCl .....	10.0g
Yeast extract .....	2.5g
Tryptone .....	2.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Nitrilotriacetic acid .....	0.1g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.04g
Phosphate buffer solution .....	100.0mL
Ferric citrate .....	0.5mL
Trace elements solution .....	0.5mL

### **Ferric Citrate Solution:**

#### **Composition per 100.0mL:**

Ferric citrate .....	0.24g
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**Preparation of Ferric Citrate Solution:** Add ferric citrate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

### **Phosphate Buffer Solution:**

#### **Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	43.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.44g

**Preparation of Phosphate Buffer Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C.

### **Trace Elements Solution:**

#### **Composition per liter:**

Nitrilotriacetic acid .....	12.8g
FeCl <sub>3</sub> ·4H <sub>2</sub> O .....	1.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.3g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.05g
H <sub>3</sub> BO <sub>3</sub> .....	0.02g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except phosphate buffer solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile phosphate buffer solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Rhodothermus marinus*.

## ***Thermus* PMY Agar (*Thermus* Peptone Meat Extract Yeast Extract Agar)**

### **Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g



Meat extract .....	3.5g
Yeast extract .....	1.5g
NaCl .....	1.5g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermus aquaticus* and other *Thermus* species.

#### *Thermus ruber* Medium

**Composition** per liter:

Agar .....	12.0g
Universal peptone .....	5.0g
Starch, soluble .....	1.0g
Yeast extract .....	1.0g
pH 8.0 ± 0.2 at 25°C	

**Source:** Universal peptone is available from Merck, Sharpe, and Dohme.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thermus ruber*.

#### *Thermus thermophilus* Medium (DSMZ Medium 74)

Polypeptone .....	8.0g
Yeast extract .....	4.0g
NaCl .....	2.0g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thermus thermophilus*.

#### *Thiobacillus* A2 Agar (T3 Agar)

**Composition** per 1100.0mL:

Solution B .....	1.0L
Solution A .....	100.0mL
pH 8.5 ± 0.2 at 25°C	

#### **Solution A:**

**Composition** per 100.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	4.2g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
Phenol Red (0.2% solution) .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 9.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **Solution B:**

**Composition** per liter:

Agar .....	15.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Trace metals solution .....	5.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 1.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **Trace Metal Solution:**

**Composition** per liter:

EDTA .....	50.0g
ZnSO <sub>4</sub> .....	22.0g
CaCl <sub>2</sub> .....	5.54g
MnCl <sub>2</sub> .....	5.06g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.99g
CoCl <sub>2</sub> .....	1.61g
CuSO <sub>4</sub> .....	1.57g
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	1.1g

**Preparation of Trace Metal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 with KOH.

**Preparation of Medium:** Aseptically add 100.0mL of sterile solution A to 1.0L of sterile solution B. Mix thoroughly. Adjust pH to 8.5 if necessary. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus versutus* and other *Thiobacillus* species.

#### *Thiobacillus acidophilus* Agar

**Composition** per liter:

Agar .....	15.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	18.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01mg
Glucose solution .....	20.0mL
pH 4.5 ± 0.2 at 25°C	

#### **Glucose Solution:**

**Composition** per 20.0mL:

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Adjust pH to 4.5 with H<sub>2</sub>SO<sub>4</sub>. Aseptically add 20.0mL of sterile glucose solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus acidophilus*.

#### *Thiobacillus acidophilus* Medium (DSMZ Medium 108)

**Composition** per liter:

Agar .....	15.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	18.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01mg
Glucose solution .....	50.0mL
pH 4.5 ± 0.2 at 25°C	

# **Glucose Solution:**

## **Composition** per 100.0mL:

D-Glucose..... 10.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 50.0mL sterile glucose solution. Mix thoroughly. Adjust pH to 4.5 with H<sub>2</sub>SO<sub>4</sub>. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Acidiphilium acidophilum*.

## ***Thiobacillus* Agar**

### **Composition** per liter:

Ionagar No. 2 ..... 12.0g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 10.0g  
K<sub>2</sub>HPO<sub>4</sub> ..... 4.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 4.0g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.1g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
CaCl<sub>2</sub> ..... 0.1g  
FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 2.0mg  
MnSO<sub>4</sub>·4H<sub>2</sub>O ..... 2.0mg

pH 6.6 ± 0.2 at 25°C

**Source:** Ionagar No. 2 is available from Oxoid Unipath.

**Preparation of Medium:** Add components, except the agar, to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Adjust the pH to 6.6. Add the agar. Bring volume to 1.0L with distilled/deionized water. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of nonaciduric *Thiobacillus* species such as *Thiobacillus neapolitanus*, *Thiobacillus novellus*, and *Thiobacillus thioparus*.

## ***Thiobacillus* Agar I for Acidophilic *Thiobacillus***

### **Composition** per liter:

Solution A ..... 500.0mL  
Solution B ..... 500.0mL

pH 4.2 ± 0.2 at 25°C

### **Solution A:**

#### **Composition** per 500.0mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> ..... 5.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 3.0g  
CaCl<sub>2</sub> ..... 0.1g  
MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.1g  
NH<sub>4</sub>Cl ..... 0.1g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 500.0mL. Adjust pH to 4.2 with 1N HCl. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

### **Solution B:**

#### **Composition** per 500.0mL:

Agar, noble..... 20.0g

**Preparation of Medium:** Aseptically combine 500.0mL of sterile solution A and 500.0mL of sterile solution B. Combine the two solutions while still hot. Mix thor-

oughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thiobacillus thiooxidans*.

## ***Thiobacillus albertis* Agar**

### **Composition** per 500.0mL:

Solution A ..... 250.0mL  
Solution B ..... 250.0mL

pH 4.0 ± 0.2 at 25°C

### **Solution A:**

#### **Composition** per 250.0mL:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 5.0g  
KH<sub>2</sub>PO<sub>4</sub> ..... 3.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.4g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.25g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 10.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Adjust pH to 4.0. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### **Solution B:**

#### **Composition** per 250.0mL:

Agar ..... 15.0g

**Preparation of Solution B:** Add agar to distilled/deionized water and bring volume to 250.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 250.0mL of sterile solution A with 250.0mL of sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thiobacillus albertis*.

## ***Thiobacillus aquaesulis* Agar**

### **Composition** per liter:

Solution B ..... 900.0mL  
Solution A ..... 100.0mL

pH 7.4 ± 0.2 at 25°C

### **Solution A:**

#### **Composition** per 100.0mL:

Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O ..... 7.9g  
KH<sub>2</sub>PO<sub>4</sub> ..... 1.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

### **Solution B:**

#### **Composition** per 900.0mL:

Agar ..... 15.0g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> ..... 5.0g  
NH<sub>4</sub>Cl ..... 0.4g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
Phenol Red ..... 3.0mg  
Trace elements solution ..... 10.0mL

### **Trace Elements Solution:**

#### **Composition** per liter:

Sodium EDTA ..... 50.0g  
NaOH ..... 11.0g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 11.0g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 7.34g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 5.0g  
MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 2.5g

CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0.

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 100.0mL of cooled, sterile solution A with 900.0mL of cooled, sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus aquaesulis*.

### *Thiobacillus Broth I* for Acidophilic *Thiobacillus*

**Composition per liter:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
CaCl <sub>2</sub> .....	0.1g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
NH <sub>4</sub> Cl .....	0.1g

pH 4.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 4.2 with 1N HCl. Mix thoroughly. Distribute into screw-capped tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus thiooxidans*.

### *Thiobacillus caldus Agar*

**Composition per liter:**

Solution E .....	500.0mL
Solution A .....	460.0mL
Solution D .....	20.0mL
Solution B .....	10.0mL
Solution C .....	10.0mL

pH 2.5 ± 0.2 at 25°C

#### **Solution A:**

**Composition per 460.0mL:**

Na <sub>2</sub> SO <sub>4</sub> ·10H <sub>2</sub> O .....	3.2g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	50.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 460.0mL. Mix thoroughly. Adjust pH to 1.75 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool and maintain above 60°C.

#### **Solution B:**

**Composition per 10.0mL:**

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	11.0mg
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	10.0mg
H <sub>3</sub> BO <sub>3</sub> .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.9mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.8mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.6mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Solution C:**

**Composition per 10.0mL:**

Glucose .....	0.45g
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**Preparation of Solution C:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### **Solution D:**

**Composition per 20.0mL:**

Na <sub>2</sub> S <sub>4</sub> O <sub>6</sub> .....	0.77g
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**Preparation of Solution D:** Add Na<sub>2</sub>S<sub>4</sub>O<sub>6</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

#### **Solution E:**

**Composition per 500.0mL:**

Phytigel™ (Gellan gum; available from Sigma Chemical Co.) .....	15.0g
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**Preparation of Solution E:** Add Phytigel to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool and maintain above 60°C.

**Preparation of Medium:** Maintain solutions A and E above 60°C to prevent rapid gelling of medium. Aseptically combine 460.0mL of sterile solution A with 10.0mL of sterile solution B, 10.0mL of sterile solution C, 20.0mL of sterile solution D, and 500.0mL of sterile solution E. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the heterotrophic cultivation and maintenance of *Thiobacillus caldus*.

### *Thiobacillus caldus Broth*

**Composition per liter:**

Solution A .....	970.0mL
Solution C .....	20.0mL
Solution B .....	10.0mL

pH 2.5 ± 0.2 at 25°C

#### **Solution A:**

**Composition per 970.0mL:**

Na <sub>2</sub> SO <sub>4</sub> ·10H <sub>2</sub> O .....	3.2g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
KCl .....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	50.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 970.0mL. Mix thoroughly. Adjust pH to 1.75 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B:**

**Composition per 10.0mL:**

FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	11.0mg
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	10.0mg
H <sub>3</sub> BO <sub>3</sub> .....	2.0mg
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	2.0mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.9mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.8mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.6mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.5mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

#### Solution C:

**Composition** per 20.0mL:

Na<sub>2</sub>S<sub>4</sub>O<sub>6</sub>..... 0.77g

**Preparation of Solution C:** Add Na<sub>2</sub>S<sub>4</sub>O<sub>6</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 970.0mL of sterile solution A with 10.0mL of sterile solution B and 20.0mL of sterile solution C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the chemolithotrophic cultivation of *Thiobacillus caldus*.

#### *Thiobacillus caldus* Medium

**Composition** per liter:

Solution E ..... 500.0mL  
Solution A ..... 460.0mL  
Solution D ..... 20.0mL  
Solution B ..... 10.0mL  
Solution C ..... 10.0mL

pH 2.5 ± 0.2 at 25°C

#### Solution A:

**Composition** per 460.0mL:

Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O..... 3.2g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>..... 3.0g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
KCl..... 0.1g  
K<sub>2</sub>HPO<sub>4</sub>..... 50.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 460.0mL. Mix thoroughly. Adjust pH to 1.75 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°–65°C.

#### Solution B:

**Composition** per 10.0mL:

FeCl<sub>3</sub>·6H<sub>2</sub>O..... 11.0mg  
Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O..... 10.0mg  
H<sub>3</sub>BO<sub>3</sub>..... 2.0mg  
MnSO<sub>4</sub>·H<sub>2</sub>O ..... 2.0mg  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.9mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 0.8mg  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.6mg  
CuSO<sub>4</sub>·5H<sub>2</sub>O ..... 0.5mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Warm to 60°C.

#### Solution C:

**Composition** per 10.0mL:

Glucose ..... 0.45g

**Preparation of Solution C:** Add glucose to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Warm to 60°C.

#### Solution D:

**Composition** per 20.0mL:

Sodium tetrathionate..... 0.77g

**Preparation of Solution D:** Add sodium tetrathionate to distilled/deionized water and bring volume to 60.0mL. Mix thoroughly. Filter sterilize. Warm to 60°C.

#### Solution E:

**Composition** per 500.0mL:

Phytigel..... 15.0g

**Preparation of Solution E:** Add Phytigel to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°–65°C.

**Preparation of Medium:** Aseptically combine 460.0mL of sterile solution A, 10.0mL of sterile solution B, 10.0mL of sterile solution C, 20.0mL of sterile solution D, and 500.0mL of sterile solution E. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thiobacillus caldus*.

#### *Thiobacillus cuprinus* Medium

**Composition** per 1010.0mL:

MgSO<sub>4</sub>·7H<sub>2</sub>O..... 3.45g  
MgCl<sub>2</sub>·6H<sub>2</sub>O..... 2.75g  
NH<sub>4</sub>Cl ..... 1.25g  
NaCl ..... 0.5g  
Sulfur, powdered ..... 0.5g  
KCl ..... 0.33g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.14g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.14g  
NiCl<sub>2</sub>·6H<sub>2</sub>O..... 2.0mg  
Trace elements solution..... 10.0mL

pH 3.5 ± 0.2 at 25°C

**Preparation of Sulfur:** Sterilize 1.0g of powdered sulfur by steaming for 1 hr on 3 consecutive days.

#### Trace Elements Solution:

**Composition** per liter:

MgSO<sub>4</sub>·7H<sub>2</sub>O..... 3.0g  
Nitrilotriacetic acid..... 1.5g  
NaCl ..... 1.0g  
MnSO<sub>4</sub>·2H<sub>2</sub>O..... 0.5g  
CoSO<sub>4</sub>·7H<sub>2</sub>O..... 0.18g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.1g  
FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O..... 0.02g  
CuSO<sub>4</sub>·5H<sub>2</sub>O..... 0.01g  
H<sub>3</sub>BO<sub>3</sub>..... 0.01g  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O..... 0.01g  
NiCl<sub>2</sub>·6H<sub>2</sub>O..... 0.025g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except sulfur, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.5 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.5g of sterile sulfur. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiobacillus cuprinus*.

#### *Thiobacillus denitrificans* Medium

**Composition** per liter:

KNO<sub>3</sub>..... 5.0g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O..... 5.0g  
NaHCO<sub>3</sub> ..... 1.0g

K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
MgCl <sub>2</sub> .....	0.1g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus denitrificans*.

#### *Thiobacillus ferrooxidans* Medium

**Composition** per liter:

Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·12H <sub>2</sub> O .....	1.4g
NaCl .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
CaCl <sub>2</sub> .....	0.03g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O solution.....	100.0mL

#### **FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:**

**Composition** per 100.0mL:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0g
H <sub>2</sub> SO <sub>4</sub> , concentrated .....	0.09mL

**Preparation of FeSO<sub>4</sub>·7H<sub>2</sub>O Solution:** Add 10.0g of FeSO<sub>4</sub>·7H<sub>2</sub>O and 0.09mL of H<sub>2</sub>SO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except FeSO<sub>4</sub>·7H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into flasks in 90.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 10.0mL of sterile FeSO<sub>4</sub>·7H<sub>2</sub>O solution to each flask. Mix thoroughly.

**Use:** For the cultivation of *Thiobacillus ferrooxidans*.

#### *Thiobacillus ferrooxidans* Medium

**Composition** per liter:

Solution I.....	400.0mL
Solution III.....	400.0mL
Solution II .....	200.0mL

#### **Solution I:**

**Composition** per 500.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
H <sub>2</sub> SO <sub>4</sub> (1N solution) .....	5.0mL

**Preparation of Solution I:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

#### **Solution II:**

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	167.0g
1N H <sub>2</sub> SO <sub>4</sub> .....	50.0mL

**Preparation of Solution II:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Warm to 45°–50°C.

#### **Solution III:**

**Composition** per liter:

Agar .....	10.0g
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**Preparation of Solution III:** Add agar to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Aseptically combine 400.0mL of sterile solution I, 200.0mL of sterile solution II, and 400.0mL of sterile solution III. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of *Thiobacillus ferrooxidans*.

#### *Thiobacillus ferrooxidans* Medium, APH

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	40.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
KCl .....	0.1g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g

pH 3.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.0 with dilute H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Leptospirillum ferrooxidans* and *Thiobacillus ferrooxidans*.

#### *Thiobacillus ferrooxidans* Medium with Ferrous Sulfate

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	33.3g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.4g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.4g
0.1 N H <sub>2</sub> SO <sub>4</sub> .....	1000.0mL

pH 1.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 1.4 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Clostridium acetobutylicum*.

#### *Thiobacillus ferrooxidans* Medium with Tetrathionate

**Composition** per liter:

K <sub>2</sub> S <sub>4</sub> O <sub>6</sub> .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g

pH 4.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4.4 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thiobacillus ferrooxidans*.

#### *Thiobacillus ferrooxidans* Medium with Thiosulfate

**Composition** per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g

pH 4.4 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 4.4 with H<sub>2</sub>SO<sub>4</sub>. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*.

#### *Thiobacillus halophilus* Agar

**Composition** per liter:

NaCl .....	50.0g
Agar .....	15.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	7.9g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Phenol Red .....	
(saturated aqueous solution) .....	12.5mL
Trace metals solution .....	10.0mL
pH 7.3 ± 0.2 at 25°C	

**Trace Metals Solution:**

**Composition** per liter:

Sodium EDTA .....	50.0g
NaOH .....	11.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Metals Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 6.0. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus halophilus*.

#### *Thiobacillus halophilus* Medium

**Composition** per liter:

Solution B .....	900.0mL
Solution A .....	100.0mL
pH 7.3 ± 0.2 at 25°C	

**Solution B:**

**Composition** per 900.0mL:

NaCl .....	50.0g
Agar, noble .....	15.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
NH <sub>4</sub> Cl .....	0.4g
Phenol Red .....	10.0mg
Hutner's basal salts solution .....	20.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Hutner's Basal Salts Solution:**

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	29.7g
Nitrilotriacetic acid .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	3.335g

FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	99.0mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	9.25mg
"Metals 44" .....	50.0mL

**"Metals 44":**

**Composition** per 100.0mL:

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.095g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
Sodium EDTA .....	0.25g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.154g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	39.2mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	24.8mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions. Add remaining components. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Solution A:**

**Composition** per 100.0mL:

Na <sub>2</sub> HPO <sub>4</sub> .....	6.3g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 900.0mL of sterile solution B with 100.0mL of sterile solution A. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Thiobacillus halophilus*.

#### *Thiobacillus* Heterotrophic Medium

**Composition** per liter:

Glucose .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.15g
KH <sub>2</sub> PO <sub>4</sub> .....	0.1g
KCl .....	0.05g
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.01g
pH 3.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Use:** For the cultivation and maintenance of *Thiobacillus organoparus* and other heterotrophic *Thiobacillus* species.

#### *Thiobacillus intermedius* Medium

**Composition** per 1010.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
Solution I .....	1.0L
Solution II .....	10.0mL

**Solution I:**

**Composition** per liter:

NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> .....	0.3g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Solution I:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution II:

**Composition** per liter:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.09g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
MnSO <sub>4</sub> .....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .....	0.01g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	5.0mg

**Preparation of Solution II:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine solution I, 10.0mL of solution II, and 10.0g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and autotrophic cultivation of *Thiobacillus intermedius*.

#### *Thiobacillus intermedius* Medium

**Composition** per 1010.0mL:

Glucose .....	10.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
Solution I .....	1.0L
Solution II .....	10.0mL

#### Solution I:

**Composition** per liter:

NH <sub>4</sub> Cl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g

**Preparation of Solution I:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution II:

**Composition** per liter:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.09g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04g
MnSO <sub>4</sub> .....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .....	0.01g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	5.0mg

**Preparation of Solution II:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine 1.0L of solution I, 10.0mL of solution II, 10.0g of glucose, and 10.0g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and mixotrophic cultivation of *Thiobacillus intermedius*.

#### *Thiobacillus* Medium

**Composition** per 100.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.1g

NH <sub>4</sub> Cl .....	0.1g
MgCl <sub>2</sub> ·7H <sub>2</sub> O .....	0.05g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus thio-parus* and *Thiobacillus thiooxidans*.

#### *Thiobacillus* Medium

**Composition** per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
CaCl <sub>2</sub> .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.02g

pH 6.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into flasks in 100.0mL volumes. Autoclave for 60 min at 0 psi pressure–100°C on 3 consecutive days.

**Use:** For the cultivation of nonaciduric *Thiobacillus* species.

#### *Thiobacillus* Medium

**Composition** per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	7.9g
Sodium formate .....	6.8g
Glucose .....	3.6g
KNO <sub>3</sub> .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Trace metals solution .....	5.0mL

pH 7.6–8.5 at 25°C

#### Trace Metals Solution:

**Composition** per liter:

Disodium EDTA .....	50.0g
NaOH .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.2g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Metals Solution:** Add EDTA to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.0 with NaOH. Add remaining components, one by one. Maintain the pH at 6.0. After dissolution of all the salts, adjust the pH to 4.0 with HCl. Store at 4°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.6–8.5. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and anaerobic cultivation of *Thiobacillus* species.

### *Thiobacillus* Medium

#### Composition per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O .....	7.9g
Sodium formate .....	6.8g
Glucose .....	3.6g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	0.3g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Trace metals solution .....	5.0mL

pH 7.6–8.5 at 25°C

#### Trace Metals Solution:

##### Composition per liter:

Disodium EDTA .....	50.0g
NaOH .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.2g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Metals Solution:** Add EDTA to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.0 with NaOH. Add remaining components, one by one. Maintain the pH at 6.0. After dissolution of all the salts, adjust the pH to 4.0 with HCl. Store at 4°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.6–8.5. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and aerobic cultivation of *Thiobacillus* species.

### *Thiobacillus* Medium B

#### Composition per liter:

Noble agar .....	15.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
NH <sub>4</sub> Cl .....	0.1g
MgCl <sub>2</sub> .....	0.1g
CaCl <sub>2</sub> .....	0.1g

pH 4.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus thiooxidans* and *Streptomyces scabies*.

### *Thiobacillus neapolitanus* Medium

#### Composition per 1002.0mL:

Solution I .....	1.0L
Solution II .....	2.0mL

pH 6.2–7.0 at 25°C

#### Solution I:

##### Composition per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.8g
KHCO <sub>3</sub> .....	0.7g
NH <sub>4</sub> Cl .....	0.4g

**Preparation of Solution I:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Solution II:

##### Composition per liter:

Disodium EDTA .....	50.0g
NaOH .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.2g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Solution II:** Add EDTA to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.0 with NaOH. Add remaining components, one by one. Maintain the pH at 6.0. After dissolution of all the salts, adjust the pH to 4.0 with HCl. Store at 4°C.

**Preparation of Medium:** Aseptically combine 1.0L of solution I and 2.0mL of solution II. Mix thoroughly. Adjust pH to 6.2–7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Thiobacillus neapolitanus*.

### *Thiobacillus novellus* Medium

#### Composition per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.3g
Yeast extract .....	0.3g
Trace metals solution .....	10.0mL

pH 6.8–7.2 at 25°C

#### Trace Metals Solution:

##### Composition per liter:

Disodium EDTA .....	50.0g
NaOH .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.5g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	2.2g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Metals Solution:** Add EDTA to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.0 with NaOH. Add remaining components, one by one. Maintain the pH at 6.0. After dissolution of all the salts, adjust the pH to 4.0 with HCl. Store at 4°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of *Thiobacillus novellus*.

### *Thiobacillus perometabolis* Medium

#### Composition per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	10.0g
Yeast extract .....	5.0g



NH <sub>4</sub> Cl.....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.6g
MgCl <sub>2</sub> .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
MgSO <sub>4</sub> .....	0.3g
Bromothymol Blue.....	0.03g
FeCl <sub>3</sub> .....	0.02g
Heavy metal solution.....	30.0mL

pH 3.0–4.0 at 25°C

**Heavy Metal Solution:****Composition per liter:**

Ethylenediamine tetraacetate.....	1.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.2g
ZnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.02g
Modified Hoagland trace elements solution.....	6.0mL

**Preparation of Heavy Metal Solution:** Add ethylenediamine tetraacetate to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.8 to dissolve EDTA. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Adjust pH to 6.8.

**Preparation of Heavy Metal Solution:** Add ethylenediamine tetraacetate to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.8 to dissolve EDTA. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly. Adjust pH to 6.8.

**Modified Hoagland Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	11.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	7.0g
AlCl <sub>3</sub> .....	1.0g
CoCl <sub>2</sub> .....	1.0g
CuCl <sub>2</sub> .....	1.0g
KI.....	1.0g
NiCl <sub>2</sub> .....	1.0g
ZnCl <sub>2</sub> .....	1.0g
BaCl <sub>2</sub> .....	0.5g
KBr.....	0.5g
LiCl.....	0.5g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.5g
SeS <sub>2</sub> .....	0.5g
SnCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.5g
NaVO <sub>3</sub> ·H <sub>2</sub> O.....	0.1g

**Preparation of Modified Hoagland Trace Elements Solution:** Add components sequentially to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3–4. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. A flaky yellow precipitate forms after mixing but will change in a few days to a fine, white precipitate. Mix the medium thoroughly before use.

**Use:** For the cultivation of *Thiobacillus perometabolis* and other bacteria that can utilize thiosulfate as an energy source.

***Thiobacillus plumbophilus* Medium****Composition per 1010.0mL:**

NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.45g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.75g

NH <sub>4</sub> Cl.....	1.25g
NaCl.....	0.5g
KCl.....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5mg
Trace elements solution.....	10.0mL

pH 6.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0 with H<sub>2</sub>SO<sub>4</sub>. Distribute 20.0mL volumes into 100.0mL serum bottles. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Check pH of medium after autoclaving and bring to 6.0 if necessary. After inoculation add 3% sterile air (9.0mL per serum bottle) with a syringe. Pressurize bottles to 2 bar with 80% H<sub>2</sub> + 20% CO<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Thiobacillus plumbophilus*.

***Thiobacillus prosperus* Medium****Composition per 1010.0mL:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.45g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.75g
NH <sub>4</sub> Cl.....	1.25g
NaCl.....	0.5g
Sulfur, powdered.....	0.5g
KCl.....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.14g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	2.0mg
Trace elements solution.....	10.0mL

pH 2.5 ± 0.2 at 25°C

**Preparation of Sulfur:** Add 10.0g of powdered sulfur to a flask and sterilize by steaming for 3 hr on 3 consecutive days.

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

**Preparation of Medium:** Add components, except sulfur, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 2.5 with H<sub>2</sub>SO<sub>4</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 0.5g of sterile sulfur. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiobacillus prosperus*.

#### *Thiobacillus tepidarius* Medium

**Composition per liter:**

Agar .....	10.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	4.96g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.8g
NH <sub>4</sub> Cl .....	0.4g
Phosphate solution .....	100.0mL
Bromocresol Purple (saturated solution) .....	2.0mL
Trace metals A-5 .....	1.0mL

#### **Phosphate Solution:**

**Composition per 100.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g

**Preparation of Phosphate Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Trace Metals A-5:**

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	49.4mg

**Preparation of Trace Metals A-5:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except phosphate solution, to distilled/deionized water and bring volume to 900.0mL. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of the sterile phosphate solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiobacillus tepidarius*.

#### *Thiobacillus/Thermophilus* Medium

**Composition per liter:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
NaHCO <sub>3</sub> .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.2g

MgCl <sub>2</sub> .....	0.1g
NH <sub>4</sub> Cl .....	0.1g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 20 min at 6 psi pressure–109°C or filter sterilize.

**Use:** For the cultivation of *Thiobacillus* species and *Thermophilus* species.

#### *Thiobacillus thiooxidans* Medium

**Composition per liter:**

Sulfur, powdered .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
FeSO <sub>4</sub> .....	0.01g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except sulfur, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into flasks in 100.0mL volumes. Add 1.0g of sulfur to each flask. Autoclave for 30 min at 0 psi pressure–100°C on 3 consecutive days.

**Use:** For the cultivation of *Thiobacillus thiooxidans*.

#### *Thiobacillus thioparus* Agar

**Composition per liter:**

Agar, purified .....	12.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	4.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
CaCl <sub>2</sub> .....	0.1g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.02g

pH 6.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.6. Add agar. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 20 min at 10 psi pressure–115°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus thioparus*.

#### *Thiobacillus thioparus* II Medium (DSMZ Medium 486)

**Composition per 1003mL:**

Solution A .....	900.0mL
Solution B .....	100.0mL
Vitamin solution .....	3.0mL

pH 7.4 ± 0.2 at 25°C

#### **Solution A:**

**Composition per 900mL:**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O .....	5.0g
NH <sub>4</sub> Cl .....	0.4g
Na <sub>2</sub> CO <sub>3</sub> .....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Bromocresol Purple (saturated solution) .....	2.0mL
Trace metals solution .....	1.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix

thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Solution B:

##### Composition per 100mL:

KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Trace Elements Solution:

##### Composition per liter:

Na <sub>2</sub> -EDTA.....	50.0g
NaOH.....	11.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	2.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Riboflavin.....	20.0mg
Ca-pantothenate.....	20.0mg
Nicotinic acid.....	20.0mg
Pyridoxine-HCl.....	20.0mg
p-Aminobenzoic acid.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	10.0mg
Biotin.....	1.0mg
Vitamin B <sub>12</sub> .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 100.0mL solution A, 900.0mL solution B, and 3.0mL vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiobacillus thioparusii*.

#### *Thiobacillus thioparusii* Agar

##### Composition per 1003.0mL:

Solution B.....	900.0mL
Solution A.....	100.0mL
Vitamin solution.....	3.0mL

pH 7.1 ± 0.2 at 25°C

#### Solution A:

##### Composition per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Solution B:

##### Composition per 900.0mL:

Agar.....	15.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	5.0g
Na <sub>2</sub> CO <sub>3</sub> .....	0.4g

NH <sub>4</sub> Cl.....	0.4g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g
Bromocresol Purple (saturated aqueous solution).....	2.0mL
Trace elements solution.....	1.0mL

#### Trace Elements Solution:

##### Composition per liter:

Disodium EDTA.....	50.0g
NaOH.....	11.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	5.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	2.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>24</sub> ·4H <sub>2</sub> O.....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0.

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Vitamin Solution:

##### Composition per liter:

Calcium DL-pantothenate.....	20.0mg
Nicotinic acid.....	20.0mg
Pyridoxine-HCl.....	20.0mg
Riboflavin.....	20.0mg
p-Aminobenzoic acid.....	10.0mg
Thiamine-HCl.....	10.0mg
Biotin.....	1.0mg
Vitamin B <sub>12</sub> .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Filter sterilize.

**Preparation of Medium:** Aseptically combine 100.0mL of sterile solution A with 900.0mL of sterile solution B and 10.0mL of sterile vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus thioparusii*.

#### *Thiobacillus thiasiris* Agar

##### Composition per 1010.0mL:

Solution A.....	100.0mL
Solution B.....	900.0mL
Vitamin solution.....	10.0mL

pH 7.3–7.6 at 25°C

#### Solution A:

##### Composition per 100.0mL:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O.....	7.9g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.4. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Solution B:

##### Composition per 900.0mL:

NaCl.....	25.1g
Agar.....	15.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	5.0g

NH <sub>4</sub> Cl .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Phenol Red .....	3.0mg
Trace elements solution .....	10.0mL

#### Trace Elements Solution:

##### Composition per liter:

Disodium EDTA .....	50.0g
NaOH .....	11.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> MoO <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.0.

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Vitamin Solution:

##### Composition per liter:

Calcium DL-pantothenate .....	20.0mg
Nicotinic acid .....	20.0mg
Pyridoxine-HCl .....	20.0mg
Riboflavin .....	20.0mg
<i>p</i> -Aminobenzoic acid .....	10.0mg
Thiamine-HCl .....	10.0mg
Biotin .....	1.0mg
Vitamin B <sub>12</sub> .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Filter sterilize.

**Preparation of Medium:** Aseptically combine 100.0mL of sterile solution A with 900.0mL of sterile solution B and 10.0mL of sterile vitamin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiobacillus thyasiris*.

#### *Thiobacillus thyasiris*/ *Thiobacillus halophilus* Medium (DSMZ Medium 484)

##### Composition per 1010mL:

Solution B .....	900.0mL
Solution A .....	100.0mL
Vitamin solution .....	10.0mL
pH 7.3–7.6 at 25°C	

#### Solution A:

##### Composition per 100mL:

Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	7.9g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Adjust pH to 7.6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Solution B:

##### Composition per 900mL:

NaCl .....	25.1g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	5.0g

NH <sub>4</sub> Cl .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
Phenol Red .....	3.0mg
Trace elements solution .....	10.0mL

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Trace Elements Solution:

##### Composition per liter:

Na <sub>2</sub> -EDTA .....	50.0g
NaOH .....	11.0g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	11.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	7.34g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	5.0g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.5g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin Solution:

##### Composition per liter:

Riboflavin .....	20.0mg
Ca-pantothenate .....	20.0mg
Nicotinic acid .....	20.0mg
Pyridoxine-HCl .....	20.0mg
<i>p</i> -Aminobenzoic acid .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	10.0mg
Biotin .....	1.0mg
Vitamin B <sub>12</sub> .....	1.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 100.0mL solution A, 900.0mL solution B, and 10.0mL vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiobacillus thyasiris*, *Thiobacillus halophilus*, *Thiomicrospira thyasirae*, and *Halotheobacillus halophilus*.

#### *Thiocapsa halophila* Medium

##### Composition per 1061.0mL:

NaCl .....	70.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	4.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
NH <sub>4</sub> Cl .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05g
NaHCO <sub>3</sub> solution .....	40.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution .....	10.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL
Trace elements solution .....	1.0mL
pH 7.2 ± 0.2 at 25°C	

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO <sub>3</sub> .....	5.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:****Composition** per 10.0mL:Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.6g**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.**Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution :****Composition** per 10.0mL:Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 0.5g**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.**Vitamin B<sub>12</sub> Solution:****Composition** per 10.0mL:Vitamin B<sub>12</sub> ..... 0.2mg**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.**Trace Elements Solution SL-12B:****Composition** per liter:

MnCl<sub>2</sub>·2H<sub>2</sub>O ..... 50.0g  
 Disodium EDTA ..... 3.0g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.1g  
 H<sub>3</sub>BO<sub>3</sub> ..... 0.3g  
 CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.19g  
 ZnCl<sub>2</sub> ..... 42.0mg  
 NiCl<sub>2</sub>·6H<sub>2</sub>O ..... 24.0mg  
 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 18.0mg  
 CuCl<sub>2</sub>·2H<sub>2</sub>O ..... 2.0mg

**Preparation of Trace Elements Solution SL-12B:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, Na<sub>2</sub>S·9H<sub>2</sub>O solution, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, and Vitamin B<sub>12</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature while sparging with 90% N<sub>2</sub> + 10% CO<sub>2</sub>. Aseptically and anaerobically add 40.0mL of sterile NaHCO<sub>3</sub> solution, 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution, 10.0mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, and 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.**Use:** For the cultivation and maintenance of *Thiocapsa halophila*.***Thiocapsa* Medium****Composition** per 127.0mL:

Solution 1 ..... 76.2mL  
 Solution 2 + Solution 3 ..... 44.8mL  
 Solution 4 ..... 6.0mL

**Solution 1:****Composition** per 2.5L:

NaCl ..... 39.68g  
 CaCl<sub>2</sub> ..... 2.0g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 2.5L. Distribute in 80.0mL volumes into 127.0mL screw-capped bottles. Autoclave for 15 min at 15 psi pressure–121°C.**Solution 2:****Composition** per 100.0mL:

Sodium ascorbate ..... 2.4g  
 KCl ..... 1.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g  
 MgCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.8g  
 NH<sub>4</sub>Cl ..... 0.8g  
 Heavy metal solution ..... 50.0mL  
 Vitamin solution ..... 15.0mL  
 Vitamin B<sub>12</sub> solution ..... 3.0mL

**Preparation of Solution 2:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.**Heavy Metal Solution:****Composition** per liter:

Ethylenediamine tetraacetate (EDTA) ..... 1.5g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g  
 ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.02g  
 Modified Hoagland trace elements solution ..... 6.0mL

**Preparation of Heavy Metal Solution:** Dissolve EDTA in approximately 800.0mL of distilled/deionized water. Add remaining components. Bring volume to 1.0L with distilled/deionized water. Mix thoroughly.**Modified Hoagland Trace Elements Solution:****Composition** per 3.6L:

H<sub>3</sub>BO<sub>3</sub> ..... 11.0g  
 MnCl<sub>2</sub>·4H<sub>2</sub>O ..... 7.0g  
 AlCl<sub>3</sub> ..... 1.0g  
 CoCl<sub>2</sub> ..... 1.0g  
 CuCl<sub>2</sub> ..... 1.0g  
 KI ..... 1.0g  
 NiCl<sub>2</sub> ..... 1.0g  
 ZnCl<sub>2</sub> ..... 1.0g  
 BaCl<sub>2</sub> ..... 0.5g  
 KBr ..... 0.5g  
 LiCl ..... 0.5g  
 Na<sub>2</sub>MoO<sub>4</sub> ..... 0.5g  
 SeCl<sub>4</sub> ..... 0.5g  
 SnCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.5g  
 NaVO<sub>3</sub>·H<sub>2</sub>O ..... 0.1g

**Preparation of Modified Hoagland Trace Elements Solution:** Prepare each component as a separate solution. Dissolve each salt in approximately 100.0mL of distilled/deionized water. Adjust the pH of each solution to below 7.0. Combine all the salt solutions and bring the volume to 3.6L with distilled/deionized water. Adjust the pH to 3–4. A yellow precipitate may form after mixing. After a few days, it will turn into a fine white precipitate. Mix the solution thoroughly before using.**Vitamin Solution:****Composition** per 100.0mL:

Pyridoxamine·2HCl ..... 5.0mg  
 Nicotinic acid ..... 2.0mg  
 Thiamine ..... 1.0mg  
 Pantothenic acid ..... 0.5mg  
 Biotin ..... 0.2mg  
*p*-Aminobenzoic acid ..... 0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.**Vitamin B<sub>12</sub> Solution:****Composition** per 100.0mL:Vitamin B<sub>12</sub> (cyanocobalamin) ..... 2.0mg**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

**Solution 3:****Composition** per 900.0mL:

NaHCO<sub>3</sub> ..... 4.5g

**Preparation of Solution 3:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Bubble 100% CO<sub>2</sub> through the solution for 30 min. After CO<sub>2</sub> saturation of solution 3, add solution 2 and immediately filter the mixture through a Seitz filter (or a Millipore) using positive CO<sub>2</sub> pressure to push the liquid through.

**Solution 4:****Composition** per 200.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 3.0g

**Preparation of Solution 4:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 200.0mL. Add a magnetic stir bar to the flask. Autoclave for 15 min at 15 psi pressure–121°C. On a magnetic stirrer, slowly add 2.0mL of sterile 2M H<sub>2</sub>SO<sub>4</sub>. This partially neutralizes the solution. The solution should turn yellow. H<sub>2</sub>S gas will be liberated; neutralization and distribution of the solution should be done as rapidly as possible under adequate ventilation.

**Preparation of Medium:** To the 80.0mL of sterile solution 1 in screw-capped bottles, add combined solutions 2 and 3 immediately after filtration and fill bottles to capacity. Mix thoroughly. Aseptically remove 6.0mL of the medium from the bottles and replace it with 6.0mL of neutralized solution 4. Let stand for 24 hr. The medium should form a fine white precipitate before using. To inoculate, remove 6.0mL of the completed medium from the bottles and replace it with 6.0mL of inoculum.

**Use:** For the cultivation and maintenance of a variety of *Thiocapsa* species.

**Thiocyanate Agar****Composition** per liter:

Solution A ..... 800.0mL

Solution B ..... 100.0mL

Solution C ..... 100.0mL

**Solution A:****Composition** per 800.0mL:

Agar, noble ..... 30.0g

K<sub>2</sub>HPO<sub>4</sub> ..... 1.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 1.0g

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

CaCl<sub>2</sub> ..... 20.0mg

FeCl<sub>3</sub>·6H<sub>2</sub>O (60%) ..... 0.1mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Solution B:****Composition** per 100.0mL:

KCNS ..... 3.6g

**Preparation of Solution B:** Add KCNS to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Solution C:****Composition** per 100.0mL:

Disodium succinate ..... 1.5g

**Preparation of Solution C:** Add disodium succinate to distilled/deionized water and bring volume to 100.0mL.

Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 800.0mL of solution A with 100.0mL of solution B and 100.0mL of solution C. Mix thoroughly. Pour into sterile Petri dishes or aseptically distribute into sterile tubes.

**Use:** For the cultivation and maintenance of a variety of microorganisms that can utilize thiocyanate as the sole source of nitrogen and sulfur.

**Thiocyanate Utilization Medium****Composition** per 1225.0mL:

Basal solution ..... 1.0L

Solution C ..... 200.0mL

Solution B ..... 20.0mL

Solution A ..... 5.0mL

**Basal Solution:****Composition** per liter:

Na<sub>2</sub>HPO<sub>4</sub> ..... 4.8g

KH<sub>2</sub>PO<sub>4</sub> ..... 4.4g

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g

**Preparation of Basal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution A:****Composition** per 100.0mL:

FeCl<sub>3</sub>·6H<sub>2</sub>O ..... 1.0g

CaCl<sub>2</sub> ..... 0.1g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution B:****Composition** per 100.0mL:

D-Glucose ..... 10.0g

**Preparation of Solution B:** Add glucose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Solution C:****Composition** per 200.0mL:

NaSCN ..... 1.0g

**Preparation of Solution C:** Add NaSCN to distilled/deionized water and bring volume to 200.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** To 1.0L of cooled, sterile basal solution, aseptically add 5.0mL of sterile solution A, 20.0mL of sterile solution B, and 200.0mL of sterile solution C. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of a variety of microorganisms that can utilize thiocyanate as the sole source of nitrogen and sulfur.

**Thioglycolate Medium  
(DSMZ Medium 530)****Composition** per liter:

Agar ..... 15.0g

Peptone from casein ..... 5.0g

Meat extract ..... 3.0g

Na-thioglycolate solution ..... 100.0mL

pH 5.5 ± 0.2 at 25°C

**Thioglycolate Solution :****Composition** per 100mL:

Na-Thioglycolate ..... 0.5g

**Preparation of Thioglycolate Solution:** Add thioglycolate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Warm to 50°C.

**Preparation of Medium:** Add components, except thioglycolate solution to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 100.0mL warm thioglycolate solution. Adjust pH to 5.5. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of various anaerobic bacteria.

***Thiomicrospira denitrificans* Agar****Composition** per 1001.0mL:

Solution A ..... 940.0mL

Solution B ..... 40.0mL

Solution C ..... 20.0mL

Solution D ..... 1.0mL

pH 7.0 ± 0.2 at 25°C

**Solution A:****Composition** per 940.0mL:

Agar ..... 15.0g

KH<sub>2</sub>PO<sub>4</sub> ..... 2.0gKNO<sub>3</sub> ..... 2.0gNH<sub>4</sub>Cl ..... 1.0gMgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.8g

Trace elements solution SL-4 ..... 2.0mL

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0 with NaOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Trace Elements Solution SL-4:****Composition** per liter:

EDTA ..... 0.5g

FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.2g

Trace elements solution SL-6 ..... 100.0

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution SL-6:****Composition** per liter:H<sub>3</sub>BO<sub>3</sub> ..... 0.3gCoCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.2gZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.1gMnCl<sub>2</sub>·4H<sub>2</sub>O ..... 0.03gNa<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O ..... 0.03gNiCl<sub>2</sub>·6H<sub>2</sub>O ..... 0.02gCuCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Solution B:****Composition** per 40.0mL:Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 5.0g

**Preparation of Solution B:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 40.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Solution C:****Composition** per 20.0mL:NaHCO<sub>3</sub> ..... 1.0g

**Preparation of Solution C:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Solution D:****Composition** per liter:FeSO<sub>4</sub>·7H<sub>2</sub>O ..... 2.0mgH<sub>2</sub>SO<sub>4</sub> (0.1N solution) ..... 1.0mL

**Preparation of Solution D:** Add FeSO<sub>4</sub>·7H<sub>2</sub>O to 1.0mL of 0.1N H<sub>2</sub>SO<sub>4</sub> solution. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Aseptically add 40.0mL of sterile solution B, 20.0mL of sterile solution C, and 1.0mL of sterile solution D to 940.0mL of sterile solution A. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes under 100% N<sub>2</sub>.

**Use:** For the cultivation and maintenance of *Thiomicrospira denitrificans*.

***Thiomicrospira* Medium  
(ATCC Medium 1036)****Composition** per liter:

NaCl ..... 25.0g

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 8.0gMgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.5g(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0gK<sub>2</sub>HPO<sub>4</sub> ..... 0.5gCaCl<sub>2</sub> ..... 0.3gVitamin B<sub>12</sub> ..... 15.0µg

Vishniac and Santer trace metals ..... 0.2mL

Bromocresol Purple (0.05% solution) ..... 0.1mL

pH 7.2 ± 0.2 at 25°C

**Vishniac and Santer Trace Metals:****Composition** per liter:

Ethylenediamine tetraacetic acid (EDTA) ..... 50.0g

ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 22.0gCaCl<sub>2</sub> ..... 5.54gMnCl<sub>2</sub>·4H<sub>2</sub>O ..... 5.06gFeSO<sub>4</sub>·7H<sub>2</sub>O ..... 4.99gCoCl<sub>2</sub>·6H<sub>2</sub>O ..... 1.61gCuSO<sub>4</sub>·5H<sub>2</sub>O ..... 1.57g(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O ..... 1.1g**Preparation of Vishniac and Santer Trace Metals:**

Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 6.0 with KOH. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiomicrospira* species.

***Thiomicrospira pelophila* Medium****Composition** per 1000.2mL:

NaCl ..... 25.0g

MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.5g(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 1.0gCaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.42g

Bromothymol Blue ..... 4.0mg

K<sub>2</sub>HPO<sub>4</sub> solution ..... 100.0mLNa<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O solution ..... 100.0mLVitamin B<sub>12</sub> solution ..... 10.0mL

Trace elements solution .....	0.2mL
Na <sub>2</sub> CO <sub>3</sub> solution .....	variable
pH 7.2 ± 0.2 at 25°C	

**K<sub>2</sub>HPO<sub>4</sub> Solution:****Composition** per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution :****Composition** per 10.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> · 5H <sub>2</sub> O .....	5.0g
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**Preparation of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Solution:** Add Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Vitamin B<sub>12</sub> Solution:****Composition** per 10.0mL:

Vitamin B <sub>12</sub> .....	15.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Trace Elements Solution:****Composition** per liter:

Disodium EDTA .....	50.0g
ZnSO <sub>4</sub> · 7H <sub>2</sub> O .....	22.0g
CaCl <sub>2</sub> · 2H <sub>2</sub> O .....	5.54g
MnCl <sub>2</sub> · 4H <sub>2</sub> O .....	5.06g
FeSO <sub>4</sub> · 7H <sub>2</sub> O .....	5.0g
CoCl <sub>2</sub> · 6H <sub>2</sub> O .....	1.61g
CuSO <sub>4</sub> · 5H <sub>2</sub> O .....	1.57g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> · 4H <sub>2</sub> O .....	1.1g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Na<sub>2</sub>CO<sub>3</sub> Solution:****Composition** per 100.0mL:

Na <sub>2</sub> CO <sub>3</sub> .....	0.4g
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**Preparation of Na<sub>2</sub>CO<sub>3</sub> Solution:** Add Na<sub>2</sub>CO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except K<sub>2</sub>HPO<sub>4</sub> solution, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5H<sub>2</sub>O solution, Vitamin B<sub>12</sub> solution, and Na<sub>2</sub>CO<sub>3</sub> solution, to distilled/deionized water and bring volume to 790.0mL. Mix thoroughly. Adjust pH to 7.2. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 100.0mL of sterile K<sub>2</sub>HPO<sub>4</sub> solution, 100.0mL of sterile Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5H<sub>2</sub>O solution, and 10.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Aseptically adjust pH to 7.2 with the appropriate volume of sterile Na<sub>2</sub>CO<sub>3</sub> solution. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiomicrospira pelophila*.

**Thiorhodococcus Medium**  
(DSMZ Medium 28a)

**Composition** per 5.0L:

Solution A .....	4.0L
Solution B .....	860.0mL
Solution E .....	100.0mL
Solution F .....	20.0mL

Solution C .....	5.0mL
Solution D .....	5.0mL
pH 7.3 at 25°C	

**Solution A:****Composition** per 4.0L:

NaCl .....	100.0g
MgCl <sub>2</sub> · 6H <sub>2</sub> O .....	5.0g
MgSO <sub>4</sub> .....	2.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> · 5H <sub>2</sub> O .....	2.5g
KH <sub>2</sub> PO <sub>4</sub> .....	1.7g
NH <sub>4</sub> Cl .....	1.7g
KCl .....	1.7g
CaCl <sub>2</sub> · 2H <sub>2</sub> O .....	1.25g

**Preparation of Solution A:** Add components to 4.0L distilled water. Mix thoroughly. Autoclave for 45 min at 15 psi pressure–121°C in 5-liter special bottle or flask with 4 openings at the top, together with a teflon-coated magnetic bar. In this 5-liter bottle, 2 openings for tubes are in the central, silicon rubber stopper. One is a short, gas-inlet tube with a sterile cotton filter; and the other is an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps. One of these openings is for the addition of sterile solutions and the other serves as a gas outlet. After autoclaving cool solution A to room temperature under a 100% N<sub>2</sub> atmosphere with a positive pressure of 0.05–0.1 atm (a manometer for low pressure will be required). Saturate the cold medium with CO<sub>2</sub> by magnetic stirring for 30 min under a CO<sub>2</sub> atmosphere of 0.05–0.1 atm.

**Solution B:**

Distilled water .....	860.0mL
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**Preparation of Solution B:** Autoclave distilled water for 15 min at 15 psi pressure–121°C in a cotton-stoppered Erlenmeyer flask. Cool to room temperature under an atmosphere of N<sub>2</sub> in an anaerobic jar.

**Solution C:****Composition** per 100.0mL:

Vitamin B <sub>12</sub> .....	2.0mg
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**Preparation of Solution C:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge under 100% N<sub>2</sub> gas for 3 min. Filter sterilize. Store under N<sub>2</sub> gas.

**Solution D:****Composition** per liter:

Disodium ethylenediamine-tetraacetate (Disodium EDTA) .....	3.0g
FeSO <sub>4</sub> · 7H <sub>2</sub> O .....	1.1g
H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> · 6H <sub>2</sub> O .....	0.19g
MnCl <sub>2</sub> · 2H <sub>2</sub> O .....	50.0mg
ZnCl <sub>2</sub> .....	42.0mg
NiCl <sub>2</sub> · 6H <sub>2</sub> O .....	24.0mg
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O .....	18.0mg
CuCl <sub>2</sub> · 2H <sub>2</sub> O .....	2.0mg

**Preparation of Solution D:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Solution E:****Composition** per 100.0mL:

NaHCO <sub>3</sub> .....	7.5g
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**Preparation of Solution E:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100%  $\text{CO}_2$  until saturated. Filter sterilize under 100%  $\text{CO}_2$  into a sterile, gas-tight 100.0mL screw-capped bottle.

#### Solution F:

**Composition** per 100.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  ..... 10.0g

**Preparation of Solution F:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### Neutralized Sulfide Solution:

**Composition** per 100.0mL:

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  ..... 1.5g

**Preparation of Neutralized Sulfide Solution:** Add  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  to distilled/deionized water in a 250.0mL screw-capped bottle fitted with a butyl rubber septum and bring volume to 100.0mL. Add a magnetic stir bar. Mix thoroughly. Sparge under 100%  $\text{N}_2$  gas for 3 min. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Adjust pH to about 7.3 with sterile 2M  $\text{H}_2\text{SO}_4$ . Do not open the bottle to add  $\text{H}_2\text{SO}_4$ ; use a sterile syringe. Stir the solution continuously to avoid precipitation of elemental sulfur. The final solution should be clear and yellow.

**Preparation of Medium:** Add solutions B, C, D, E, and F to solution A through one of the screw-capped openings against a stream of either  $\text{N}_2$  gas or better, a mixture of 95%  $\text{N}_2$  and 5%  $\text{CO}_2$  while the medium is magnetically stirred. Adjust the pH of the medium with sterile HCl or  $\text{Na}_2\text{CO}_3$  solution (2 M solutions) to pH 7.3. Distribute the medium aseptically through the medium outlet tube into sterile, 100mL bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05–0.1 atm) of the  $\text{N}_2/\text{CO}_2$  gas mixture. Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-capped bottles can be stored for several weeks or months in the dark. During the first 24 hr, the iron of the medium precipitates in the form of black flocs. No other sediment should arise in the otherwise clear medium. Incubate in the light at 500–1000 lux intensity. Feed periodically with neutralized solution of sodium sulfide to replenish sulfide and with other supplement solutions.

**Use:** For the cultivation of *Thiorhodococcus minor*.

#### *Thiosphaera Agar*

**Composition** per liter:

Agar ..... 15.0g  
 $\text{Na}_2\text{HPO}_4$  ..... 4.2g  
 $\text{KH}_2\text{PO}_4$  ..... 1.5g  
 $\text{NH}_4\text{Cl}$  ..... 0.3g  
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g  
 $\text{KNO}_3$  ..... 0.1g  
 Vishniac and Santer trace metals ..... 2.0mL  
 pH 8.0–8.2 at 25°C

#### Vishniac and Santer Trace Metals:

**Composition** per liter:

Ethylenediamine tetraacetic acid (EDTA) ..... 50.0g  
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 22.0g  
 $\text{CaCl}_2$  ..... 5.54g  
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 5.06g  
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 4.99g  
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 1.61g

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ..... 1.57g  
 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  ..... 1.1g

#### Preparation of Vishniac and Santer Trace Metals:

Add components to distilled/deionized water and bring volume to 1.0L. Adjust pH to 6.0 with KOH. Mix thoroughly.

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 8.0–8.2. Filter sterilize. Warm to 45°–50°C. Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically combine the two sterile solutions. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiosphaera pantotropha*.

#### *Thiosphaera pantotropha* Medium

**Composition** per 1001.0mL:

Agar ..... 20.0g  
 $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  ..... 7.9g  
 $\text{KH}_2\text{PO}_4$  ..... 1.5g  
 $\text{NH}_4\text{Cl}$  ..... 0.3g  
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g  
 Yeast extract solution ..... 10.0mL  
 Trace elements solution SL-10 ..... 1.0mL  
 pH 7.5 ± 0.2 at 25°C

#### Yeast Extract Solution:

**Composition** per 10.0mL:

Yeast extract ..... 1.0g

**Preparation of Yeast Extract Solution:** Add yeast extract to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution SL-10:

**Composition** per liter:

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 1.5g  
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 190.0mg  
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ..... 100.0mg  
 $\text{ZnCl}_2$  ..... 70.0mg  
 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  ..... 36.0mg  
 $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  ..... 24.0mg  
 $\text{H}_3\text{BO}_3$  ..... 6.0mg  
 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  ..... 2.0mg  
 HCl (25% solution) ..... 10.0mL

#### Preparation of Trace Elements Solution SL-10:

Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except yeast extract solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile yeast extract solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Paracoccus denitrificans*.

#### Thiosulfate-Oxidizing Medium

**Composition** per liter:

$\text{K}_2\text{HPO}_4$  ..... 2.0g  
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ..... 0.1g

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	0.02g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> solution.....	100.0mL
Thiosulfate solution.....	100.0mL

pH 7.8 ± 0.2 at 25°C

#### (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Solution:

**Composition** per 100.0mL:

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
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**Preparation of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Solution:** Add the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C

#### Thiosulfate Solution:

**Composition** per 100.0mL:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	10.0g
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**Preparation of Thiosulfate Solution:** Add the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C.

**Preparation of Medium:** Add components, except (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution and thiosulfate solution, to distilled/deionized water and bring volume to 800.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add the sterile (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution and the sterile thiosulfate solution. Mix thoroughly. Adjust the pH to 7.8 if necessary. Aseptically distribute into sterile tubes or flasks.

**Use:** For the isolation and cultivation of iron and sulfur bacteria.

#### Thiosulfate Salts Broth

**Composition** per liter:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	24.81g
NH <sub>4</sub> Cl.....	2.2g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
Artificial seawater.....	500.0mL

#### Artificial Seawater:

**Composition** per liter:

NaCl.....	23.476g
MgCl <sub>2</sub> .....	4.981g
Na <sub>2</sub> SO <sub>4</sub> .....	3.917g
CaCl <sub>2</sub> .....	1.102g
KCl.....	0.664g
NaHCO <sub>3</sub> .....	0.192g
KBr.....	0.096g
H <sub>3</sub> BO <sub>3</sub> .....	0.026g
SrCl <sub>2</sub> .....	0.024g
NaF.....	3.0mg

pH 5.0 ± 0.2 at 25°C

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 5.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thiobacillus* species.

#### Thiothrix Agar (DSMZ Medium 573)

**Composition** per liter:

Agar.....	12.0g
NH <sub>4</sub> Cl.....	0.2g
Na-acetate.....	0.1g

K <sub>2</sub> HPO <sub>4</sub> .....	0.01g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01g
CaSO <sub>4</sub> (saturated solution).....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution.....	5.0mL

pH 7.5 ± 0.2 at 25°C

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Trace Elements Solution:

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.7g
EDTA.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
H <sub>3</sub> BO <sub>3</sub> .....	10.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	2.0mg
Co(NO <sub>3</sub> ) <sub>2</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0μg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Pour into Petri dishes or aseptically distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Thiothrix nivea*.

#### Thiothrix Medium (DSMZ Medium 573)

**Composition** per liter:

NH <sub>4</sub> Cl.....	0.2g
Na-acetate.....	0.1g
K <sub>2</sub> HPO <sub>4</sub> .....	0.01g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.01g
CaSO <sub>4</sub> (saturated solution).....	20.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Trace elements solution.....	5.0mL

pH 7.5 ± 0.2 at 25°C

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

#### Trace Elements Solution:

**Composition** per liter:

FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.7g
EDTA.....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	10.0mg
H <sub>3</sub> BO <sub>3</sub> .....	10.0mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	2.0mg
Co(NO <sub>3</sub> ) <sub>2</sub> .....	1.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	1.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	5.0μg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL sterile  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Thiothrix niva*.

### Tibi Medium

**Composition per liter:**

Sucrose.....	100.0–150.0g
Fig, dried, quartered.....	1
Lemon wedge (0.5cm segment).....	1

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L in a 1.0L Erlenmeyer flask fitted with a cotton stopper. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Inoculate with about 50.0mL of Tibi grains.

**Use:** For the cultivation of osmophilic bacteria and fungi from tibi grains.

### TMA Mineral Medium

**Composition per liter:**

Agar, noble.....	20.0g
$\text{KH}_2\text{PO}_4$ .....	2.78g
$\text{Na}_2\text{HPO}_4$ .....	2.78g
$(\text{NH}_4)_2\text{SO}_4$ .....	1.0g
Tetramethylammonium perchlorate solution.....	20.0mL
Hutner's basal salts solution.....	20.0mL

pH  $6.8 \pm 0.2$  at 25°C

### Tetramethylammonium Perchlorate Solution:

**Composition per 20.0mL:**

Tetramethylammonium perchlorate.....	1.0g
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**Preparation of Tetramethylammonium Perchlorate Solution:** Add tetramethylammonium perchlorate to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

### Hutner's Basal Salts Solution:

**Composition per liter:**

$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ .....	29.7g
Nitritotriacetic acid.....	10.0g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	3.335g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	99.0mg
$(\text{NH}_4)_6\text{MoO}_7\cdot \text{O}_{24}\cdot 4\text{H}_2\text{O}$ .....	9.25mg
"Metals 44".....	50.0mL

### "Metals 44":

**Composition per 100.0mL:**

$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ .....	1.095g
$\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ .....	0.5g
Sodium EDTA.....	0.25g
$\text{MnSO}_4\cdot \text{H}_2\text{O}$ .....	0.154g
$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ .....	39.2mg
$\text{Co}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$ .....	24.8mg
$\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$ .....	17.7mg

**Preparation of "Metals 44":** Add sodium EDTA to distilled/deionized water and bring volume to 90.0mL. Mix thoroughly. Add a few drops of concentrated  $\text{H}_2\text{SO}_4$  to retard precipitation of heavy metal ions. Add remaining com-

ponents. Mix thoroughly. Bring volume to 100.0mL with distilled/deionized water.

**Preparation of Hutner's Basal Salts Solution:** Add nitritotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

**Preparation of Medium:** Add components, except tetramethylammonium perchlorate solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 20.0mL of sterile tetramethylammonium perchlorate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Paracoccus kocurii*.

### TMBS4 Medium (DSMZ Medium 559)

**Composition per 1002.0mL:**

Solution A.....	870.0mL
Solution C.....	100.0mL
Solution D.....	10.0mL
Solution E (Vitamin solution).....	10.0mL
Solution F.....	10.0mL
Solution B (Trace elements solution SL-10).....	1.0mL
Solution G.....	1.0mL
Solution H.....	1.0mL

pH 7.1–7.4 at 25°C

### Solution A:

**Composition per 870.0mL:**

$\text{NaCl}$ .....	1.0g
$\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ .....	0.4g
$\text{Na}_2\text{SO}_4$ .....	3.0g
$\text{KCl}$ .....	0.5g
$\text{NH}_4\text{Cl}$ .....	0.3g
$\text{KH}_2\text{PO}_4$ .....	0.2g
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ .....	0.15g
Resazurin.....	1.0mg

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 870.0mL. Mix thoroughly.

### Solution B (Trace Elements Solution SL-10):

**Composition per liter:**

$\text{FeCl}_2\cdot 4\text{H}_2\text{O}$ .....	1.5g
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ .....	190.0mg
$\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ .....	100.0mg
$\text{ZnCl}_2$ .....	70.0mg
$\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ .....	36.0mg
$\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ .....	24.0mg
$\text{H}_3\text{BO}_3$ .....	6.0mg
$\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ .....	2.0mg
$\text{HCl}$ (25% solution).....	10.0mL

**Preparation of Solution B (Trace Elements Solution SL-10):** Add  $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$  to 10.0mL of  $\text{HCl}$  solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100%  $\text{N}_2$ . Autoclave for 15 min at 15 psi pressure–121°C.

### Solution C:

**Composition per 100.0mL:**

$\text{NaHCO}_3$ .....	5.0g
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**Preparation of Solution C:** Add  $\text{NaHCO}_3$  to distilled/deionized water and bring volume to 100.0mL. Mix thor-

oughly. Filter sterilize. Flush with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen.

#### Solution D:

##### Composition per 10.0mL:

Syringic acid ..... 0.6g

**Preparation of Solution D:** Add syringic acid to distilled/deionized water and bring volume to 10.0mL. Adjust pH to 8.0 with NaOH. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution E (Vitamin Solution):

##### Composition per liter:

Pyridoxine-HCl ..... 10.0mg  
Thiamine-HCl·2H<sub>2</sub>O ..... 5.0mg  
Riboflavin ..... 5.0mg  
Nicotinic acid ..... 5.0mg  
D-Ca-pantothenate ..... 5.0mg  
*p*-Aminobenzoic acid ..... 5.0mg  
Lipoic acid ..... 5.0mg  
Biotin ..... 2.0mg  
Folic acid ..... 2.0mg  
Vitamin B<sub>12</sub> ..... 0.10mg

**Solution E (Vitamin Solution):** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution F:

##### Composition per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.4g

**Preparation of Solution F:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution G:

##### Composition per 10.0mL:

Na-dithionite ..... 0.25g

**Preparation of Solution G:** Add Na-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Solution H

##### Composition per liter:

NaOH ..... 0.5g  
Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O ..... 4.0mg  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O ..... 3.0mg

**Preparation of Solution H:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Gently heat solution A and bring to boiling. Boil solution A for a few minutes. Cool to room temperature. Gas with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH below 6. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Sequentially add 1.0mL solution B, 100.0mL solution C, 10.0mL solution D, 10.0mL solution E, 10.0mL solution F, 1.0mL solution G, and 1.0mL solution H. Distribute anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels.

**Use:** For the cultivation of *Holophaga foetida*.

#### *Treponema bryantii* Medium

##### Composition per liter:

L-Cysteine-HCl ..... 1.0g  
NaCl ..... 0.9g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ..... 0.9g  
K<sub>2</sub>HPO<sub>4</sub> ..... 0.45g  
KH<sub>2</sub>PO<sub>4</sub> ..... 0.45g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.18g  
CaCl<sub>2</sub>·2H<sub>2</sub>O ..... 0.12g  
Resazurin ..... 1.0mg  
Rumen fluid, clarified ..... 300.0mL  
NaHCO<sub>3</sub> solution ..... 100.0mL  
Glucose solution (10%w/v) ..... 20.0mL  
pH 7.0 ± 0.2 at 25°C

#### NaHCO<sub>3</sub> Solution:

##### Composition per 100.0mL:

NaHCO<sub>3</sub> ..... 5.0g

**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% CO<sub>2</sub>.

#### Glucose Solution:

##### Composition per 20.0mL:

Glucose ..... 2.0g

**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize. Sparge with 100% CO<sub>2</sub>.

**Preparation of Medium:** Prepare and dispense medium under 100% CO<sub>2</sub>. Add components, except rumen fluid, NaHCO<sub>3</sub> solution, and glucose solution, to distilled/deionized water and bring volume to 580.0mL. Mix thoroughly. Adjust pH to 7.0 with KOH. Sparge with 100% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 300.0mL of sterile rumen fluid, 100.0mL of sterile NaHCO<sub>3</sub> solution, and 20.0mL of sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Treponema bryantii*.

#### Triple Sugar Iron Agar (TSI Agar)

##### Composition per liter:

Peptone ..... 20.0g  
Agar ..... 12.0g  
Lactose ..... 10.0g  
Sucrose ..... 10.0g  
NaCl ..... 5.0g  
Beef extract ..... 3.0g  
Yeast extract ..... 3.0g  
Glucose ..... 1.0g  
Ferric citrate ..... 0.3g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> ..... 0.3g  
Phenol Red ..... 0.025g

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position to form a 1.0-inch butt.

**Use:** For the differentiation of members of the Enterobacteriaceae based on their fermentation of lactose, sucrose, and glucose and the production of H<sub>2</sub>S.

### Triple Sugar Iron Agar (TSI Agar)

#### Composition per liter:

Agar .....	13.0g
Pancreatic digest of casein .....	10.0g
Peptic digest of animal tissue .....	10.0g
Lactose .....	10.0g
Sucrose .....	10.0g
NaCl .....	5.0g
Glucose .....	1.0g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	0.2g
Phenol Red .....	0.025g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Allow tubes to cool in a slanted position to form a 1.0 inch butt.

**Use:** For the differentiation of members of the Enterobacteriaceae based on their fermentation of lactose, sucrose, and glucose and the production of H<sub>2</sub>S.

### Tris YP Agar (Tris Yeast Extract Peptone Agar)

#### Composition per liter:

Agar .....	19.0g
Yeast extract .....	3.0g
Glucose .....	1.0g
Peptone .....	0.6g
Tris-buffer (0.05M, pH 7.5) .....	1.0L

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. For top layer agar, add 6.0g of agar instead of 19.0g. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the cultivation and maintenance of *Bdellovibrio* species.

### Trypticase Serum Seawater Agar (ATCC Medium 1359)

#### Composition per liter:

Agar .....	12.0g
Pancreatic digest of casein .....	1.0g
Seawater .....	990.0mL
Horse serum .....	10.0mL

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except horse serum, to 990.0mL seawater. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile horse serum. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of fastidious marine bacterial species.

### Trypticase Soy Agar

#### Composition per liter:

Pancreatic digest of casein .....	17.0g
Agar .....	15.0g

NaCl .....	5.0g
Papaic digest of soybean meal .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Glucose .....	2.5g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a wide variety of heterotrophic microorganisms.

### Trypticase Soy Agar (ATCC Medium 18)

#### Composition per liter:

Pancreatic digest of casein .....	17.0g
Agar .....	15.0g
NaCl .....	5.0g
Papaic digest of soybean meal .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Glucose .....	2.5g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of a wide variety of fastidious and nonfastidious microorganisms from clinical and nonclinical specimens. Also used for the rapid estimation of the bacteriological quality of water.

### Trypticase Soy Agar (Tryptic Soy Agar) (Soybean Casein Digest Agar) (ATCC Medium 77)

#### Composition per liter:

Pancreatic digest of casein .....	15.0g
Agar .....	15.0g
Papaic digest of soybean meal .....	5.0g
NaCl .....	5.0g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Do not overheat. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of a wide variety of fastidious as well as nonfastidious microorganisms.

### Trypticase Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

#### Composition per liter:

Pancreatic digest of casein .....	15.0g
Agar .....	15.0g
Papaic digest of soybean meal .....	5.0g
NaCl .....	5.0g
Polysorbate 80 (Tween 80) .....	5.0g
Lecithin .....	0.7g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 13 psi pressure–118°C. Cool to 45°–50°C. Pour into sterile Petri dishes in 17.0mL volumes or leave in tubes.

**Use:** For the detection and enumeration of microorganisms in replicate plating techniques. Also used for the detection and enumeration of microorganisms present on surfaces of sanitary importance.

#### **Trypticase Soy Agar with NaCl (ATCC Medium 176)**

**Composition per liter:**

NaCl .....	30.0g
Pancreatic digest of casein .....	15.0g
Agar .....	15.0g
Papaic digest of soybean meal .....	5.0g
Bile salts No. 3 .....	1.0g

pH 7.3 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Vibrio alginolyticus*.

#### **Trypticase Soy Broth (Soybean Casein Digest Broth, USP) (LMG Medium 185)**

**Composition per liter:**

Pancreatic digest of casein .....	17.0g
NaCl .....	5.0g
Papaic digest of soybean meal .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Glucose .....	2.5g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of a wide variety of fastidious and nonfastidious microorganisms from nonclinical specimens. Also used for the rapid estimation of the bacteriological quality of water.

#### **Trypticase Soy Broth with Sea Salts (LMG Medium 249)**

**Composition per liter:**

Sea salts .....	20.0g
Pancreatic digest of casein .....	17.0g
NaCl .....	5.0g
Papaic digest of soybean meal .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g
Glucose .....	2.5g
Yeast extract .....	1.0

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For cultivation and maintenance of *Cellulophaga bal-tica* and *Cellulophaga fucicola*.

#### **Tryptone Soya Agar**

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of casein .....	15.0g
NaCl .....	5.0g
Pancreatic digest of soybean meal .....	5.0g

pH 7.3 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a wide variety of microorganisms.

#### **Tryptone Thioglycolate Medium (DSMZ Medium 48)**

**Composition per liter:**

Yeast extract .....	6.0g
K <sub>2</sub> HPO <sub>4</sub> .....	5.45g
Peptone .....	2.0g
Tryptone .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.2g
Na-thioglycolate .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.025g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.015g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	2.5mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	2.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.0mg
Glucose solution .....	50.0mL

pH 7.5 ± 0.2 at 25°C

**Glucose Solution:**

**Composition per 50.0mL:**

D-Glucose .....	20.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except glucose solution to distilled/deionized water and bring volume to 950.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 50.0mL sterile glucose solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Clostridium beijerinckii*.

#### **Tryptone Yeast Extract Salt Medium**

**Composition per liter:**

Solution A .....	500.0mL
Solution B .....	500.0mL

pH 6.8 ± 0.2 at 25°C

**Solution A:**

**Composition per 500.0mL:**

NaCl .....	125.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	50.0g

K <sub>2</sub> SO <sub>4</sub> .....	5.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.2g

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Solution B:**

**Composition** per 500.0mL:

Pancreatic digest of casein.....	5.0g
Yeast extract.....	5.0g

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Adjust pH to 6.8. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Aseptically combine 500.0mL of solution A with 500.0mL of solution B. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Haloferax volcanii*.

#### **TS Agar (DSMZ Medium 893)**

**Composition** per liter:

Agar .....	15.0g
Sucrose.....	5.0g
Tryptone.....	5.0g
Beef extract.....	3.0g
Glucose .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Saccharococcus thermophilus*.

#### **TS Medium for *Spirochaeta caldaria***

**Composition** per liter:

Pancreatic digest of casein.....	2.0g
Cellobiose .....	1.0g
Maltose.....	1.0g
Yeast extract.....	1.0g
Dithiothreitol.....	0.15g
Resazurin .....	1.0mg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Continue boiling for 3 min. Cool to room temperature while sparging with 100% N<sub>2</sub>. Anaerobically distribute into anaerobic tubes. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 7.0

**Use:** For the cultivation of *Spirochaeta caldaria*.

#### **TS Soil Extract (Trypticase Soy Soil Extract)**

**Composition** per liter:

Pancreatic digest of casein.....	17.0g
Agar .....	15.0g
NaCl.....	5.0g
Papaic digest of soybean meal.....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	2.5g

Glucose.....	2.5g
Soil extract.....	250.0mL

#### **Soil Extract:**

**Composition** per 400.0mL:

African Violet soil .....	154.0g
Na <sub>2</sub> CO <sub>3</sub> .....	0.4g

**Preparation of Soil Extract:** Add components to tap water and bring volume to 400.0mL. Autoclave for 60 min at 15 psi pressure–121°C. Filter through Whatman filter paper.

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus xerothermodurans*.

#### **TTD-Medium (DSMZ Medium 480b)**

**Composition** per liter:

NaCl (marine salts).....	25.0g
Sulfur, powder.....	10.0g
Casitone.....	5.0g
NH <sub>4</sub> Cl.....	0.33g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.33g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.33g
KCl.....	0.33g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33g
Resazurin.....	1.0mg
Na <sub>2</sub> S·9H <sub>2</sub> O solution.....	10.0mL
Vitamin solution.....	10.0mL
Trace elements solution SL-10.....	1.0mL

pH 6.9 ± 0.2 at 25°C

#### **Trace Elements Solution SL-10:**

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0mg
HCl (25% solution).....	10.0mL

#### **Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### **Vitamin Solution:**

**Composition** per liter:

Pyridoxine-HCl.....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O.....	5.0mg
Riboflavin.....	5.0mg
Nicotinic acid.....	5.0mg
D-Ca-pantothenate.....	5.0mg
p-Aminobenzoic acid.....	5.0mg
Lipoic acid.....	5.0mg
Biotin.....	2.0mg
Folic acid.....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix

thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

##### Composition per 10.0mL:

Na<sub>2</sub>S·9H<sub>2</sub>O ..... 0.5g

**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Sparge with N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Store anaerobically.

**Preparation of Medium:** Prepare and dispense medium under an oxygen-free 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution to 980.0mL distilled/deionized water. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Adjust pH to 5.9 with concentrated NaOH. Sterilize medium by heating for 1 hr at 90–100°C on 3 subsequent days. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Before use, aseptically and anaerobically add 10.0mL sterile vitamin solution and 10.0mL sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thermococcus stetteri*.

#### TY Salts Medium

##### Composition per liter:

Pancreatic digest of casein ..... 1.0g  
Yeast extract ..... 1.0g  
Salts solution ..... 100.0mL

pH 8.2 ± 0.2 at 25°C

#### Salts Solution:

##### Composition per liter:

NaNO<sub>3</sub> ..... 6.89g  
KNO<sub>3</sub> ..... 1.03g  
MgSO<sub>4</sub>·7H<sub>2</sub>O ..... 1.0g  
Nitrilotriacetic acid ..... 1.0g  
CaSO<sub>4</sub>·2H<sub>2</sub>O ..... 0.6g  
NaCl ..... 80.0mg  
FeCl<sub>3</sub> solution ..... 10.0mL  
Trace elements solution ..... 10.0mL

**Preparation of Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.2 with 1M NaOH. Autoclave for 15 min at 15 psi pressure–121°C.

#### FeCl<sub>3</sub> Solution:

##### Composition per 100.0mL:

FeCl<sub>3</sub> ..... 28.0mg

**Preparation of FeCl<sub>3</sub> Solution:** Add FeCl<sub>3</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly.

#### Trace Elements Solution:

##### Composition per liter:

MnSO<sub>4</sub>·H<sub>2</sub>O ..... 2.2g  
H<sub>3</sub>BO<sub>3</sub> ..... 0.5g  
ZnSO<sub>4</sub>·7H<sub>2</sub>O ..... 0.5g  
CoCl<sub>2</sub>·6H<sub>2</sub>O ..... 46.0mg  
Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O ..... 25.0mg  
CuSO<sub>4</sub> ..... 16.0mg  
H<sub>2</sub>SO<sub>4</sub> ..... 0.5mL

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components, except salts solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi

pressure–121°C. Aseptically add 100.0mL of sterile salts solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thermomonospora aquaticus*, *Thermus filiformis*, *Thermus flavus*, *Thermus ruber*, and other *Thermus* species.

#### TYN Medium

##### Composition per liter:

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O ..... 10.0g  
Pancreatic digest of casein ..... 1.0g  
Yeast extract ..... 1.0g  
Na<sub>2</sub>SO<sub>4</sub> ..... 1.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Thiobacillus* species.

#### Tyrosine Casein Nitrate Medium (TCN Medium)

##### Composition per liter:

Sodium caseinate ..... 25.0g  
Agar ..... 15.0g  
NaNO<sub>3</sub> ..... 10.0g  
L-Tyrosine ..... 1.0g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of streptomycetes from infected plants.

#### TZC Selective Medium

##### Composition per liter:

Agar ..... 17.0g  
Peptone ..... 1.0g  
Glucose ..... 0.5g  
Pancreatic digest of casein ..... 0.1g  
2,3,5-Triphenyltetrazolium·HCl solution ..... 10.0mL

#### 2,3,5-Triphenyltetrazolium·HCl Solution:

##### Composition per 10.0mL:

2,3,5-Triphenyltetrazolium·HCl ..... 0.05g

**Preparation of 2,3,5-Triphenyltetrazolium·HCl Solution:** Add 2,3,5-triphenyltetrazolium·HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except 2,3,5-triphenyltetrazolium·HCl solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add 10.0mL of sterile 2,3,5-triphenyltetrazolium·HCl solution. Mix thoroughly. Pour into sterile Petri dishes.

**Use:** For the isolation, cultivation, and differentiation of *Pseudomonas solanacearum*. The virulent, wild-type strains appear as irregular to round white colonies with a pink center. Avirulent mutants, which readily occur in nature, appear as round, deep red colonies with a narrow blue border.



**Urea Agar**  
(Urease Test Agar)  
(Urea Agar Base, Christensen)

**Composition per liter:**

Urea .....	20.0g
Agar .....	15.0g
NaCl .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	2.0g
Peptone .....	1.0g
Glucose .....	1.0g
Phenol Red .....	0.012g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components, except agar, to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize. Add agar to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add the 100.0mL of sterile basal medium. Mix thoroughly. Distribute into sterile tubes. Allow tubes to solidify in a slanted position.

**Use:** For the differentiation of a variety of microorganisms, especially members of the Enterobacteriaceae, aerobic actinomycetes, streptococci, and nonfermenting Gram-negative bacteria, on the basis of urease production.

**Urea R Broth**  
(Urea Rapid Broth)

**Composition per liter:**

Urea .....	20.0g
Yeast extract .....	0.1g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.095g
KH <sub>2</sub> PO <sub>4</sub> .....	0.091g
Phenol Red .....	0.01g

pH 6.9 ± 0.2 at 25°C

**Source:** This medium is available as a prepared medium from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the differentiation of members of the Enterobacteriaceae based on the rapid detection of urease activity. Urease-positive bacteria turn the medium cerise.

**Urea Semisolid Medium**

**Composition per liter:**

Solution A .....	400.0mL
Solution B .....	50.0mL

**Solution A:****Composition per 400.0mL:**

Pancreatic digest of casein .....	6.0g
Yeast extract .....	2.0g
NaCl .....	1.0g
Yeast extract .....	0.8g
Agar .....	0.3g
L-Cystine .....	0.1g
Thioglycollic acid .....	0.12mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 400.0mL. Mix

thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 60°C.

**Solution B:****Composition per 50.0mL:**

Urea .....	8.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	3.8g
KH <sub>2</sub> PO <sub>4</sub> .....	3.64g
Yeast extract .....	0.04g
Phenol Red .....	4.0mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 50.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 400.0mL of sterile solution A and 50.0mL of sterile solution B. Mix thoroughly. Aseptically distribute into sterile screw-capped tubes in 7.0mL volumes. Pass the tubes into an anaerobic chamber containing 85% N<sub>2</sub> + 10% H<sub>2</sub> + 5% CO<sub>2</sub> for 60 min. Close screw caps tightly.

**Use:** For the cultivation and differentiation of anaerobic bacteria based on their production of urease. Bacteria that produce urease turn the medium bright red.

**Urea Test Broth**

**Composition per liter:**

Urea .....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	9.5g
KH <sub>2</sub> PO <sub>4</sub> .....	9.1g
Yeast extract .....	0.1g
Phenol Red .....	0.01g
Urea solution .....	100.0mL

**Urea Solution:****Composition per 100.0mL:**

Urea .....	20.0g
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**Preparation of Urea Solution:** Add urea to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except urea solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile urea solution. Mix thoroughly. Aseptically distribute into sterile tubes in 3.0mL volumes.

**Use:** For the cultivation and differentiation of members of the Enterobacteriaceae and aerobic actinomycetes based on their production of urease. Bacteria that produce urease turn the medium bright red.

**Urease Test Broth**  
(Urea Broth)

**Composition per liter:**

Urea .....	20.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	9.5g
KH <sub>2</sub> PO <sub>4</sub> .....	9.1g
Yeast extract .....	0.1g
Phenol Red .....	0.01g

pH 6.8 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the differentiation of organisms, especially the Enterobacteriaceae, on the basis of urease production. Urease-positive bacteria turn the medium pink.

### ***Ustilago Complete Agar II***

**Composition** per liter:

Agar .....	20.0g
Glucose .....	10.0g
Hydrolyzed casein.....	2.5g
NH <sub>4</sub> NO <sub>3</sub> .....	1.5g
Yeast extract.....	1.0g
Salt solution .....	62.5mL
Vitamin solution.....	10.0mL
Hydrolyzed nucleic acids solution.....	5.0mL

pH 7.0 ± 0.2 at 25°C

### **Salt Solution:**

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	16.0g
KCl.....	8.0g
Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
MgSO <sub>4</sub> .....	2.0g
CaCl <sub>2</sub> .....	1.0g
Trace elements solution .....	8.0mL

### **Trace Elements Solution:**

**Composition** per liter:

CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.4g
ZnCl <sub>2</sub> .....	0.4g
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.14g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	100.0mg
H <sub>3</sub> BO <sub>3</sub> .....	60.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Vitamin Solution:**

**Composition** per liter:

Inositol .....	1.0g
Calcium pantothenate .....	0.2g
Choline chloride .....	0.2g
Nicotinic acid.....	0.2g
Thiamine .....	100.0mg
<i>p</i> -Aminobenzoic acid.....	50.0mg
Pyridoxin.....	50.0mg
Riboflavin .....	50.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Hydrolyzed Nucleic Acids Solution:**

**Composition** per 80.0mL:

DNA, calf thymus .....	2.0g
RNA .....	2.0g
HCl (1M solution).....	30.0mL
NaOH (1M solution).....	30.0mL

**Preparation of Hydrolyzed Nucleic Acids Solution:** Add DNA to 30.0mL of 1M NaOH solution. Add RNA to 30.0mL of 1M HCl solution. Autoclave the 2 solutions separately for 10 min at 15 psi pressure–121°C. Mix the two solutions. Adjust the pH to 6.0. Centrifuge at 5000 × g for 10 min. Decant supernatant solution and filter. Bring volume to 80.0mL with distilled/deionized water. Store at –20°C.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Ustilago* species.

### ***Ustilago Complete Broth II***

**Composition** per liter:

Glucose .....	10.0g
Hydrolyzed casein.....	2.5g
NH <sub>4</sub> NO <sub>3</sub> .....	1.5g
Yeast extract .....	1.0g
Salt solution.....	62.5mL
Vitamin solution .....	10.0mL
Hydrolyzed nucleic acids solution .....	5.0mL

pH 7.0 ± 0.2 at 25°C

### **Salt Solution:**

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	16.0g
KCl .....	8.0g
Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
MgSO <sub>4</sub> .....	2.0g
CaCl <sub>2</sub> .....	1.0g
Trace elements solution .....	8.0mL

### **Trace Elements Solution:**

**Composition** per liter:

CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.4g
ZnCl <sub>2</sub> .....	0.4g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.14g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	100.0mg
H <sub>3</sub> BO <sub>3</sub> .....	60.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	40.0mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Vitamin Solution:**

**Composition** per liter:

Inositol.....	1.0g
Calcium pantothenate .....	0.2g
Choline chloride .....	0.2g
Nicotinic acid .....	0.2g
Thiamine.....	100.0mg
<i>p</i> -Aminobenzoic acid .....	50.0mg
Pyridoxin .....	50.0mg
Riboflavin.....	50.0mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

### **Hydrolyzed Nucleic Acids Solution:**

**Composition** per 80.0mL:

DNA, calf thymus .....	2.0g
RNA .....	2.0g
HCl (1M solution).....	30.0mL
NaOH (1M solution).....	30.0mL

**Preparation of Hydrolyzed Nucleic Acids Solution:** Add DNA to 30.0mL of 1M NaOH solution. Add RNA to 30.0mL of 1M HCl solution. Autoclave the 2 solutions separately for 10 min at 15 psi pressure–121°C. Mix the two solutions. Adjust the pH to 6.0. Centrifuge at 5000 × g for 10

min. Decant supernatant solution and filter. Bring volume to 80.0mL with distilled/deionized water. Store at  $-20^{\circ}\text{C}$ .

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ .

**Use:** For the cultivation of *Ustilago* species.

### *Ustilago* Medium

**Composition** per liter:

Yeast extract .....	11.0g
Glucose .....	10.0g
$\text{NH}_4\text{NO}_3$ .....	1.5g
Salt solution .....	62.5mL
Vitamin solution .....	10.0mL

**Salt Solution:**

**Composition** per liter:

$\text{KH}_2\text{PO}_4$ .....	16.0g
KCl .....	8.0g
$\text{Na}_2\text{SO}_4$ .....	4.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	2.0g
$\text{CaCl}_2$ .....	1.0g
Trace elements solution .....	8.0mL

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:**

**Composition** per 500.0mL:

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .....	0.2g
$\text{ZnCl}_2$ .....	0.2g
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	0.07g
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .....	0.05g
$\text{H}_3\text{BO}_3$ .....	0.03g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Vitamin Solution:**

**Composition** per liter:

Inositol .....	0.4g
Calcium pantothenate .....	0.2g
Choline chloride .....	0.2g
Nicotinic acid .....	0.2g
Thiamine .....	0.1g
Pyridoxine .....	0.05g
Riboflavin .....	0.05g

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vitamin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ . Cool to  $45^{\circ}\text{--}50^{\circ}\text{C}$ . Aseptically add 10.0mL of sterile vitamin solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Ustilago* species.

### *Ustilago* Minimal Medium

**Composition** per liter:

Glucose .....	10.0g
$\text{KNO}_3$ .....	3.0g
Salt solution .....	62.5mL

**Salt Solution:**

**Composition** per liter:

$\text{KH}_2\text{PO}_4$ .....	16.0g
KCl .....	8.0g
$\text{Na}_2\text{SO}_4$ .....	4.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	2.0g
$\text{CaCl}_2$ .....	1.0g
Trace elements solution .....	8.0mL

**Preparation of Salt Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Trace Elements Solution:**

**Composition** per 500.0mL:

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .....	0.2g
$\text{ZnCl}_2$ .....	0.2g
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	0.07g
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .....	0.05g
$\text{H}_3\text{BO}_3$ .....	0.03g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .....	0.02g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ .

**Use:** For the cultivation of *Ustilago* species.

### V-8<sup>TM</sup> Agar

**Composition** per liter:

Agar .....	15.0g
$\text{CaCO}_3$ .....	2.0g
V-8 canned vegetable juice .....	200.0mL

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ . Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of numerous yeasts and filamentous fungi.

### V-8-0 Agar

**Composition** per liter:

Agar .....	15.0 g
$\text{CaCO}_3$ .....	5.0 g
$\text{CaCl}_2$ .....	100.0mg
$\beta$ -Sitosterol .....	30.0mg
Tryptophan .....	20.0mg
Thiamine .....	1.0mg
V-8 canned vegetable juice .....	354.0mL

**Preparation of Medium:** Add  $\text{CaCO}_3$  to V-8. Centrifuge for 20 min at 4000rpm. Decant the supernatant. Add the supernatant to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Add remaining components. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure– $121^{\circ}\text{C}$ . Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Phytophthora syringae*, *Phytophthora palmivora*, *Phytophthora nicotianae*, *Phytophthora erythroseptica*, *Phytophthora drechsleri*, and *Phytophthora citrophthora*.

# V-8 Juice Seawater Agar

## Composition per liter:

Agar .....	15.0 g
CaCO <sub>3</sub> .....	3.0 g
Artificial seawater .....	800.0 mL
V-8 canned vegetable juice, unsalted .....	200.0 mL
pH 7.0 ± 0.2 at 25°C	

## Artificial Seawater:

NaCl .....	20.0 g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.38 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	6.78 g
KCl .....	0.72 g
NaHCO <sub>3</sub> .....	0.2 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.4 g

**Preparation of Artificial Seawater:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Combine components. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Halophytophthora masteri* and *Halophytophthora tartarea*.

# V-8 Rye Agar

## Composition per 1050.0mL:

Agar .....	20.0 g
CaCO <sub>3</sub> .....	0.2 g
Rye broth .....	1.0 L
V-8 canned vegetable juice .....	50.0 mL

## Rye broth:

## Composition per liter:

Whole rye grains, .....	50.0 g
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**Preparation of Rye Broth:** Soak the rye grains in 1.1L of distilled/deionized water for 24–36 hr at 24°C. Autoclave at 15 psi pressure–121°C for 30 min. Filter through four layers of cheesecloth. Bring the final filtrate volume to 1.0L with distilled/deionized water.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Phytophthora infestans*.

# V24N Medium (DSMZ Medium 88b)

Starch, soluble .....	2.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.3g
Sulfur, powdered .....	1.0g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
KH <sub>2</sub> PO <sub>4</sub> .....	0.28g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
Yeast extract .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.07g
FeCl <sub>3</sub> ·6H <sub>2</sub> O .....	0.02g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O .....	4.5mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.8mg
Resazurin .....	0.4mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.05mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg

VOSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03mg
CoSO <sub>4</sub> .....	0.01mg
pH 6.0± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust the pH to 6.0 with H<sub>2</sub>SO<sub>4</sub> (25% v/v). Sparge medium with 100% N<sub>2</sub> gas. Distribute anaerobically into rubber stoppered tubes or bottles that have been presterilized by autoclaving. On 2 successive days heat the medium to 85 °C for 2 hr in order to sterilize.

**Use:** For the growth and maintenance of *Thermophilum librum*.

# Van Niel's Agar

## Composition per liter:

Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Rhodomicrobium vannielii*.

# Van Niel's Medium, Modified

## Composition per liter:

Yeast extract .....	10.0g
MgSO <sub>4</sub> .....	0.1g
EDTA .....	2.0mg
Trace elements solution .....	10.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	2.5mL
pH 7.1 ± 0.2 at 25°C	

## Trace Elements Solution:

## Composition per 100.0mL:

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.3g
Ferric ammonium citrate .....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

## K<sub>2</sub>HPO<sub>4</sub> Solution:

## Composition per 100.0mL:

K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except K<sub>2</sub>HPO<sub>4</sub> and trace elements solution, to distilled/deionized water and bring volume to 987.5mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add trace elements and K<sub>2</sub>HPO<sub>4</sub> solutions. Mix thoroughly. Distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of *Rhodobacter sphaeroides*.

# Van Niel's Yeast Agar with Glutamate (ATCC Medium 1370)

## Composition per liter:

Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

Glutamate.....	0.7g
MgSO <sub>4</sub> .....	0.5g
pH 7.1 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Rhodomicrobium* spp.

#### Van Niel's Yeast Agar with NaCl

<b>Composition per liter:</b>	
NaCl .....	25.0g
Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
pH 7.1 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of halophilic *Rhodomicrobium* spp.

#### Van Niel's Yeast Agar with 25% NaCl (ATCC Medium 217)

<b>Composition per liter:</b>	
NaCl .....	250.0g
Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
pH 7.1 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of halophilic *Rhodomicrobium* spp.

#### Van Niel's Yeast Agar with Succinate (ATCC Medium 1243)

<b>Composition per liter:</b>	
Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
Succinate .....	0.6g
MgSO <sub>4</sub> .....	0.5g
pH 7.1 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Rhodomicrobium* spp.

#### Van Niel's Yeast Medium with Pyruvate, Modified

<b>Composition per liter:</b>	
Yeast extract .....	10.0g
MgSO <sub>4</sub> .....	0.1g
EDTA .....	2.0mg

Sodium pyruvate solution .....	100.0mL
Trace elements solution .....	10.0mL
K <sub>2</sub> HPO <sub>4</sub> solution .....	5.0mL
Trace metal A-5 solution .....	1.0mL
pH 7.1 ± 0.1 at 25°C	

#### Sodium Pyruvate Solution:

**Composition per 100.0mL:**

Sodium pyruvate .....	1.1g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add sodium pyruvate to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Trace Elements Solution:

**Composition per 100.0mL:**

CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.3g
Ferric ammonium citrate .....	0.2g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### K<sub>2</sub>HPO<sub>4</sub> Solution:

**Composition per 100.0mL:**

K <sub>2</sub> HPO <sub>4</sub> .....	4.0g
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**Preparation of K<sub>2</sub>HPO<sub>4</sub> Solution:** Add K<sub>2</sub>HPO<sub>4</sub> to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

#### Trace Metal A-5 Solution:

**Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	2.86g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.39g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.222g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.079g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.049g

**Preparation of Trace Metal A-5 Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except sodium pyruvate, trace elements, K<sub>2</sub>HPO<sub>4</sub>, and trace metal A-5 solutions, to distilled/deionized water and bring volume to 884.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically add 100.0mL of sterile sodium pyruvate solution, 10.0mL of sterile trace elements solution, 5.0mL of sterile K<sub>2</sub>HPO<sub>4</sub> solution, and 1.0mL of sterile trace metal A-5 solution. Mix thoroughly. Adjust pH to 7.1 ± 0.1. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation and maintenance of photosynthetic bacteria, such as *Heliobacillus mobilis* and *Rhodospseudomonas palustris*.

#### Vanillate Medium

**Composition per liter:**

Agar .....	20.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.4g
Yeast extract .....	0.1g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.01g
Trace elements solution .....	10.0mL
Vanillic acid solution .....	10.0mL

#### Trace Elements Solution:

**Composition per liter:**

MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.4g
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4mg

FeCl <sub>3</sub> .....	0.2mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O .....	0.2mg
KI .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04mg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

#### Vanillic Acid Solution:

**Composition** per 10.0mL:

Vanillic acid, sodium salt .....	1.5g
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**Preparation of Vanillic Acid Solution:** Add vanillic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except vanillic acid solution and trace elements solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Warm the vanillic acid solution and the trace elements solution to 50°–55°C. Aseptically add 10.0mL of sterile vanillic acid solution and 10.0mL of sterile trace elements solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Pseudomonas fluorescens*.

#### VCR Medium

**Composition** per 1001.0mL:

Agar, noble .....	15.0g
NaNO <sub>3</sub> .....	2.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.2g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
FeCl <sub>3</sub> .....	0.01g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	15.0mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	1.0mg
Vitamin B <sub>12</sub> solution .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Vitamin B<sub>12</sub> Solution:

**Composition** per 10.0mL:

Vitamin B <sub>12</sub> .....	10.0μg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add Vitamin B<sub>12</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Vitamin B<sub>12</sub> solution, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 1.0mL of sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Sphaerobacter thermomethanica*.

#### VEN CHI2 Medium (DSMZ Medium 293a)

**Composition** per liter:

NaCl .....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	3.0g
KCl .....	0.5g
NH <sub>4</sub> Cl .....	0.25g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg

NaHCO <sub>3</sub> solution .....	10.0mL
Na <sub>2</sub> -succinate solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL
Vitamin solution .....	10.0mL
Quinic acid solution .....	10.0mL
Trace elements solution SL-10 .....	1.0mL

pH 7.2 ± 0.2 at 25°C

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.36g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

#### NaHCO<sub>3</sub> Solution:

**Composition** per 10.0mL:

NaHCO <sub>3</sub> .....	2.5g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Na<sub>2</sub>-Succinate Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> -succinate .....	3.25g
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**Preparation of Na<sub>2</sub>-Succinate Solution:** Add Na<sub>2</sub>-succinate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

#### Vitamin Solution:

**Composition** per liter:

Pyridoxine-HCl .....	10.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	5.0mg
Riboflavin .....	5.0mg
Nicotinic acid .....	5.0mg
D-Ca-pantothenate .....	5.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Quinic Acid Solution:

**Composition** per 10.0mL:

Quinic acid .....	1.0g
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**Preparation of Quinic Acid Solution:** Add quinic acid to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Adjust pH to 7.0. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Filter sterilize.

#### Trace Elements Solution SL-10:

**Composition** per liter:

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  gas atmosphere. Add components, except  $\text{NaHCO}_3$  solution,  $\text{Na}_2\text{-succinate}$  solution,  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, vitamin solution, quinic acid solution, and trace elements solution SL-10, to distilled/deionized water and bring volume to 949.0mL. Mix thoroughly. Adjust pH to 7.2. Sparge with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$ . Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 10.0mL  $\text{NaHCO}_3$  solution, 10.0mL  $\text{Na}_2\text{-succinate}$  solution, 10.0mL  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  solution, 10.0mL vitamin solution, 10.0mL quinic acid solution, and 1.0mL trace elements solution SL-10. Mix thoroughly. Aseptically and anaerobically distribute into sterile tubes or bottles. After inoculation, flush and repressurize the gas head space of culture bottles with sterile 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  to 1 bar overpressure.

**Use:** For the cultivation of *Ilyobacter insuetus*.

***Vibrio Agar*****Composition per liter:**

Sucrose.....	20.0g
Agar.....	15.0g
NaCl.....	10.0g
Sodium citrate $\cdot 2\text{H}_2\text{O}$ .....	10.0g
$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ .....	6.5g
Oxgall.....	5.0g
Yeast extract.....	5.0g
Pancreatic digest of casein.....	4.0g
Proteose peptone.....	3.0g
Sodium deoxycholate.....	1.0g
Sodium lauryl sulfate.....	0.2g
Water Blue.....	0.2g
Cresol Red.....	0.02g

pH 8.5  $\pm$  0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 8.5. Gently heat and bring to boiling. Do not autoclave. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the isolation and cultivation of the *Vibrio cholerae*.

***Vibrio costicola* Medium****Composition per liter:**

NaCl.....	81.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	19.7g
Agar.....	15.0g
$\text{MgCl}_2 \cdot \text{H}_2\text{O}$ .....	15.0g
Yeast extract.....	10.0g
Proteose peptone.....	5.0g
KCl.....	2.0g
Glucose.....	1.0g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .....	0.48g
$\text{NaHCO}_3$ .....	60.0mg
NaBr.....	26.0mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Vibrio costicola*.

***Vibrio* Medium****Composition per liter:**

NaCl.....	10.0g
Pancreatic digest of casein.....	10.0g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ .....	4.0g
KCl.....	1.0g

pH 7.5  $\pm$  0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Vibrio diazotrophicus*.

***Vibrio natriegens* Medium****Composition per liter:**

Urea.....	20.0g
NaCl.....	15.0g
Agar.....	15.0g
Peptone.....	5.0g
Meat extract.....	3.0g

pH 7.0  $\pm$  0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Vibrio natriegens*.

***Vibrio parahaemolyticus* Agar  
(VP Agar)****Composition per liter:**

Agar.....	20.0g
NaCl.....	20.0g
Sucrose.....	20.0g
Sodium citrate.....	10.0g
$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ .....	10.0g
Peptone.....	10.0g
Sodium taurocholate.....	5.0g
Yeast extract.....	5.0g
Sodium lauryl sulfate.....	0.2g
Bromothymol Blue.....	0.04g
Thymol Blue.....	0.04g

pH 8.6  $\pm$  0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not autoclave. Pour into sterile Petri dishes.

**Use:** For the isolation, cultivation, enumeration, and presumptive identification of coliforms in specimens of sanitary significance. Sucrose-fermenting bacteria appear as yellow colonies with pale yellow peripheries. Sucrose-non-fermenting bacteria appear as mucoid, green colonies with a dark green center.

***Vibrio vallismortis* Medium****Composition per liter:**

NaCl.....	25.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	9.6g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ .....	7.0g
Glucose.....	5.0g
KCl.....	3.8g
Yeast extract.....	1.0g

CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O.....	0.4g
NaHCO <sub>3</sub> solution.....	20.0mL
pH 7.0 ± 0.2 at 25°C	

**NaHCO<sub>3</sub> Solution:****Composition per 20.0mL:**

NaHCO <sub>3</sub> .....	3.0g
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**Preparation of NaHCO<sub>3</sub> Solution:** Add NaHCO<sub>3</sub> to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except NaHCO<sub>3</sub> solution, to distilled/deionized water and bring volume to 980.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature. Aseptically add 20.0mL of sterile NaHCO<sub>3</sub> solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Vibrio vallismortis*.

**Violet Peptone Bile Lactose Broth****Composition per liter:**

Lactose.....	10.0g
Peptone.....	10.0g
Bile salts.....	5.0g
Gentian Violet.....	0.04g

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the selective cultivation of members of the Enterobacteriaceae.

**Violet Red Bile Agar (VRB Agar)****Composition per liter:**

Agar.....	15.0g
Lactose.....	10.0g
Pancreatic digest of gelatin.....	7.0g
NaCl.....	5.0g
Yeast extract.....	3.0g
Bile salts.....	1.5g
Neutral Red.....	0.03g
Crystal Violet.....	2.0mg

pH 7.4 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour immediately into sterile Petri dishes or leave in tubes.

**Use:** For the detection of coliform bacteria in water.

**VM1 Medium (DSMZ Medium 890)****Composition per liter:**

NaCl.....	20.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	12.6g
Na <sub>2</sub> SO <sub>4</sub> .....	3.24g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.38g
KCl.....	0.56g
Sulfur, powdered.....	0.5g

NH <sub>4</sub> Cl.....	0.3g
K <sub>2</sub> HPO <sub>4</sub> .....	0.2g
NaHCO <sub>3</sub> .....	0.16g
Trace elements solution.....	10.0mL

pH 7.0 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	3.0g
Nitrilotriacetic acid.....	1.5g
NaCl.....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O.....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.18g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.1g
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.025g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O.....	0.02g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O.....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O.....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O.....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Dissolve by adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0 using H<sub>2</sub>SO<sub>4</sub>. Fill 20.0mL medium into 100mL serum bottles and seal with a rubber stopper. Add atmosphere of 78% H<sub>2</sub> + 20% CO<sub>2</sub> + 2% O<sub>2</sub> with an overpressure. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the autotrophic cultivation of *Hydrogenothermus marinus*.

**Von Hofsten & Malmquist Medium B****Composition per liter:**

Agar.....	15.0g
NaNO <sub>3</sub> .....	2.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
CaCl <sub>2</sub> ·H <sub>2</sub> O.....	0.02g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.02g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.02g
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	0.02g

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Alteromonas* species and *Cytophaga saccharophila*.

**VP Medium (Voges-Proskauer Medium)****Composition per liter:**

Peptone.....	7.0g
K <sub>2</sub> HPO <sub>4</sub> .....	5.0g
Glucose.....	5.0g

pH 6.9 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.9. Distribute into tubes in 3.0mL volumes. Autoclave for 15 min at 15 psi pressure–121°C.



**Use:** For the cultivation and differentiation of bacteria based on their ability to produce acetoin.

### VY Agar

#### Composition per liter:

Agar .....	15.0g
Baker's yeast .....	10.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
Cyanocobalamin .....	5.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of myxobacteria.

### VY2 Agar

#### Composition per liter:

Agar .....	15.0g
Baker's yeast .....	5.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
Cyanocobalamin .....	5.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Myxococcus amylovorans*.

### VY5 Agar

#### Composition per liter:

Agar .....	15.0g
Baker's yeast .....	2.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
Cyanocobalamin .....	5.0mg

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of myxobacteria.

### VY/4-SWS Agar (DSMZ Medium 958)

#### Composition per 1001mL:

NaCl .....	20.0g
Agar .....	15.0g
Yeast cell paste (Baker's yeast, washed in deionized water) wet weight .....	2.5g
Seawater salts solution .....	1000.0mL
Vitamin B <sub>12</sub> solution .....	1.0mL

pH 7.5 ± 0.2 at 25°C

#### Seawater Salts Solution:

##### Composition per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	8.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
KCl .....	0.5g
NaHCO <sub>3</sub> .....	0.16g
KBr .....	0.08g
SrCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.03g
H <sub>3</sub> BO <sub>3</sub> .....	0.02g

Ferric citrate .....	0.01g
di-Na-β-glycerophosphate .....	0.01g
Trace elements solution SL-4 .....	1.0mL

#### Trace Elements Solution SL-4:

##### Composition per liter:

EDTA .....	0.5g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution SL-6 .....	100.0mL

**Preparation of Trace Elements Solution SL-4:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Trace Elements Solution SL-6:

##### Composition per liter:

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution SL-6:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 3.4.

**Preparation of Seawater Salts Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

#### Vitamin B<sub>12</sub> Solution:

##### Composition per 10.0mL:

Cyanocobalamin .....	5.0mg
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**Preparation of Vitamin B<sub>12</sub> Solution:** Add cyanocobalamin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Add NaCl, agar, and yeast cell paste to 1.0L seawater salts solution. Mix thoroughly. Adjust pH to 7.5 with 1M NaOH. Gently heat and bring to boiling. Autoclave for 20 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 1.0mL sterile Vitamin B<sub>12</sub> solution. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Haliangium ochraceum*, *Haliangium tepidum*, *Plesiocystis pacifica*, and *Enhygromyxa salina* (*Thaxteria salina*).

### W Medium

#### Composition per liter:

Sulfur .....	10.0g
KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0mg

pH 3.0 ± 0.2 at 25°C

**Preparation of Sulfur:** Sterilize by steaming at 100°C for 60 min on 3 consecutive days.

**Preparation of Medium:** Add components, except sulfur, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0g of sterile sulfur. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Thiobacillus thiooxidans*.

**Waksman's Glucose Agar****Composition** per liter:

Agar .....	12.5g
Glucose .....	10.0g
Peptone.....	5.0g
Beef extract.....	5.0g
NaCl .....	5.0g

pH 7.4-7.6 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Streptomyces* species.

**Waksman's Sulfur Medium****Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	3.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.2g
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	0.1mg

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. It is not necessary to sterilize this medium. Distribute into sterile tubes or flasks.

**Use:** For the cultivation of sulfate-reducing microorganisms from soil.

**Walsby Medium****Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.075g
K <sub>2</sub> HPO <sub>4</sub> .....	0.039g
Na <sub>2</sub> CO <sub>3</sub> .....	0.02g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.018g
H <sub>3</sub> BO <sub>3</sub> .....	2.8mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	2.0mg
ZnSO <sub>4</sub> .....	0.22mg
MoO <sub>3</sub> .....	0.18mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.08mg
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.05mg
Iron-EDTA solution .....	1.0mL

pH 8.5 ± 0.2 at 25°C

**Iron-EDTA Solution:****Composition** per liter:

EDTA .....	12.7g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	4.98g

**Preparation of Iron-EDTA Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of planktonic gas-vacuolate cyanobacteria.

**Water Agar****Composition** per liter:

Agar .....	15.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of myxobacteria.

**Waxy Maize Starch Medium****Composition** per liter:

Agar .....	20.0g
Waxy maize starch.....	5.0g
Pancreatic digest of casein .....	5.0g
Yeast extract .....	5.0g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
Maltose solution .....	100.0mL

pH 6.7 ± 0.2 at 25°C

**Maltose Solution:****Composition** per 100.0mL:

Maltose .....	10.0g
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**Preparation of Maltose Solution:** Add maltose to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except maltose solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.7. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add maltose solution. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus* species.

**WCX Agar****Composition** per liter:

Agar .....	15.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	1.0g
Cycloheximide solution.....	100.0mL

pH 7.2 ± 0.2 at 25°C

**Cycloheximide Solution****Composition** per 100.0mL:

Cycloheximide .....	2.5mg
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**Preparation of Cycloheximide Solution:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except cycloheximide solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile cycloheximide solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of myxobacteria.

**Wilbrinck Agar for *Xanthomonas*****Composition** per liter:

Sucrose .....	20.0g
Agar .....	12.0g
Peptone .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas caryophylli*, *Xanthomonas albilineans*, and *Xanthomonas axonopodis*.

### Wilbrinck Agar for *Xanthomonas albilineans*

#### Composition per liter:

Agar .....	20.0g
Sucrose .....	10.0g
Peptone .....	5.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
Na <sub>2</sub> SO <sub>3</sub> , anhydrous .....	0.05g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas albilineans* and other *Xanthomonas* species.

### Winogradsky's Medium, Modified

#### Composition per liter:

CaCO <sub>3</sub> .....	5.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NaCl .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
FeSO <sub>4</sub> .....	0.4g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat until dissolved. Do not autoclave. Distribute into tubes or flasks. Swirl flask while dispensing to suspend precipitate.

**Use:** For the cultivation of nitrifying bacteria.

### Winogradsky's N-Free Medium

#### Composition per liter:

Agar .....	20.0g
CaCO <sub>3</sub> .....	5.0mg
Sugar solution .....	100.0mL
Concentrated salt solution .....	5.0mL

pH 7.2 ± 0.2 at 25°C

#### Sugar Solution:

##### Composition per 100.0mL:

Sucrose or glucose .....	10.0g
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**Preparation of Sugar Solution:** Add sucrose or glucose to 100.0mL of distilled/deionized water. Mix thoroughly. Autoclave for 10 min at 10 psi pressure–115°C. Cool to 50°C.

#### Concentrated Salt Solution:

##### Composition per liter:

KH <sub>2</sub> PO <sub>4</sub> .....	50.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	25.0g
NaCl .....	25.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	1.0g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	1.0g
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	1.0g

**Preparation of Concentrated Salt Solution:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except sugar solution, to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add sugar solution. Adjust pH to 7.2. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Azomonas insignis*.

### Winogradsky's Nitrite Medium

#### Composition per liter:

Agar .....	15.0g
NaNO <sub>2</sub> .....	2.0g
Na <sub>2</sub> CO <sub>3</sub> , anhydrous .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the selective isolation and cultivation of *Nocardia* species and *Rhodococcus* species.

### WMC Medium

#### Composition per 1010.0mL:

NaHCO <sub>3</sub> .....	5.0g
Sodium acetate .....	1.0g
L-Cysteine .....	0.5g
Resazurin .....	1.0mg
Mineral salt solution 2xW .....	500.0mL
LIP solution .....	50.0mL
TYC solution .....	50.0mL
Trace elements solution .....	10.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	10.0mL

pH 7.2 ± 0.2 at 25°C

#### Mineral Solution 2xW:

##### Composition per liter:

NaCl .....	40.0g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	5.6g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.7g
KCl .....	0.68g
NH <sub>4</sub> Cl .....	0.5g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.28g
K <sub>2</sub> HPO <sub>4</sub> .....	0.28g

**Preparation of Mineral Solution 2xW:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize. Store at room temperature in the dark.

#### LIP Solution:

##### Composition per liter:

L-Isoleucine .....	10.0g
L-Leucine .....	5.0g
Pantothenate .....	0.1g

**Preparation of LIP Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. May be stored unsterilized at -20°C.

#### TYC Solution:

##### Composition per liter:

Casamino acids .....	100.0g
Yeast extract .....	50.0g
L-Tryptophan .....	1.0g

**Preparation of TYC Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

#### Trace Elements Solution:

**Composition** per liter:

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·2H <sub>2</sub> O .....	0.5g
CoSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.18g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.02g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	0.3mg

**Preparation of Trace Elements Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjusting pH to 6.5 with KOH. Add remaining components. Add distilled/deionized water to 1.0L.

#### Na<sub>2</sub>S·9H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.5g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Add components, except TYC solution and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 940.0mL. Mix thoroughly. Sparge with 80% H<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 50.0mL of sterile TYC solution and 10.0mL of sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thoroughly.

**Use:** For the cultivation and maintenance of *Methanococcus voltae*.

#### Woods and Welton Agar

**Composition** per liter:

NaCl .....	23.4g
Casein hydrolysate .....	17.0g
Agar .....	15.0g
Glycerol .....	10.0g
Pancreatic digest of gelatin .....	5.0g
Glucose .....	5.0g
Papaic digest of soybean meal .....	3.0g
Beef extract .....	3.0g
Yeast extract .....	2.0g
Casamino acids, vitamin free .....	0.5g
Pancreatic digest of casein .....	0.5g
Na <sub>2</sub> SO <sub>3</sub> .....	0.1g

pH 7.6 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.6. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Vibrio alginolyticus*.

#### Xanthine Agar

**Composition** per liter:

Solution 1 .....	900.0mL
Solution 2 .....	100.0mL

pH 7.0 ± 0.2 at 25°C

#### Solution 1:

**Composition** per 900.0mL:

Agar .....	15.0g
Pancreatic digest of gelatin .....	5.0g
Beef extract .....	3.0g

**Preparation of Solution 1:** Add components to distilled/deionized water and bring volume to 900.0mL. Mix thoroughly. Gently heat and bring to boiling.

#### Solution 2:

**Composition** per 100.0mL:

Xanthine .....	4.0g
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**Preparation of Solution 2:** Add xanthine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Gently heat and bring to boiling.

**Preparation of Medium:** Combine solutions 1 and 2. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the differentiation of aerobic *Actinomycete* species. Clearing around a colony indicates utilization of xanthine. *Streptomyces* species utilize xanthine; most *Nocardia* and *Actinomadura* species do not utilize xanthine.

#### Xanthobacter agilis Agar

**Composition** per 1100.0mL:

Solution A .....	1.0L
Solution B .....	100.0mL

#### Solution A:

**Composition** per liter:

Agar .....	15.0g
NaH <sub>2</sub> PO <sub>4</sub> ·12H <sub>2</sub> O .....	9.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.5g
NH <sub>4</sub> Cl .....	1.0g
Sodium propionate or 3-hydroxybutyrate .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
Trace elements solution .....	1.0mL

pH 7.0 ± 0.2 at 25°C

#### Trace Elements Solution:

**Composition** per 2.0mL:

H <sub>3</sub> BO <sub>4</sub> .....	560.0μg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	350.0μg
NiCl <sub>2</sub> ·H <sub>2</sub> O .....	160.0μg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	100.0μg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	16.0μg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	16.0μg

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 2.0mL. Mix thoroughly.

**Preparation of Solution A:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

#### Solution B:

**Composition** per 100.0mL:

Ferric ammonium citrate .....	50.0mg
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	100.0mg

**Preparation of Solution B:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix

thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 1.0L of sterile solution A with 100.0mL of sterile solution B. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Xanthobacter agilis*, a dinitrogen-fixing, hydrogen-oxidizing bacterium.

#### *Xanthomonas Agar*

**Composition per liter:**

Agar .....	15.0g
Pancreatic digest of gelatin .....	10.0g
Sucrose .....	10.0g
Beef extract .....	6.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas* species.

#### *Xanthomonas albilineans Agar*

**Composition per liter:**

Sucrose .....	20.0g
Agar .....	15.0g
Peptone .....	10.0g
Yeast extract .....	5.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Xanthomonas albilineans*.

#### *Xanthomonas maltophilia Medium*

**Composition per liter:**

Trizma® (tris[Hydroxymethyl]aminomethane) base .....	6.04g
Glucose .....	5.0g
KCl .....	1.0g
NaCl .....	1.0g
L-Phenylalanine .....	0.9g
MgSO <sub>4</sub> .....	0.2g
L-Arginine .....	0.1g
L-Methionine .....	0.1g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1g
NH <sub>4</sub> Cl .....	0.1g
Glycerol .....	0.68g
L-Serine .....	0.22g
L-Alanine .....	0.18g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Filter sterilize. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Stenotrophomonas maltophilia*.

#### *Xanthomonas Medium*

**Composition per liter:**

Pancreatic digest of gelatin .....	10.0g
Sucrose .....	10.0g

Beef extract .....	6.0g
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pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat with mixing. Distribute into screw-capped test tubes. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation and maintenance of *Xanthomonas* species.

#### *Xanthomonas TYG Agar* (*Xanthomonas* Tryptone Yeast Extract Glucose Agar)

**Composition per liter:**

Agar .....	20.0g
Pancreatic digest of casein .....	5.0g
Glucose .....	5.0g
Yeast extract .....	3.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.7g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas* species.

#### *Xenorhabdus Agar*

**Composition per liter:**

Agar .....	15.0g
Peptone .....	10.0g
NaCl .....	5.0g
Yeast extract .....	5.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Distribute into tubes or flasks. Autoclave for 20 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacteroides galacturonicus* and *Xenorhabdus nematophilus*.

#### XLD Agar (Xylose Lysine Deoxycholate Agar)

**Composition per liter:**

Agar .....	13.5g
Lactose .....	7.5g
Sucrose .....	7.5g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .....	6.8g
L-Lysine .....	5.0g
NaCl .....	5.0g
Xylose .....	3.5g
Yeast extract .....	3.0g
Sodium deoxycholate .....	2.5g
Ferric ammonium citrate .....	0.8g
Phenol Red .....	0.08g

pH 7.5 ± 0.2 at 25°C

**Source:** This medium is available as a premixed powder from BD Diagnostic Systems and Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Do not overheat. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

Plates should be poured as soon as possible to avoid precipitation.

**Use:** For the isolation and differentiation of enteric pathogens, especially *Shigella* and *Providencia* species. Nonfermenting xylose/lactose/sucrose bacteria appear as red colonies. Xylose-fermenting, lysine-decarboxylating bacteria appear as red colonies. Xylose-fermenting, lysine-non-decarboxylating bacteria appear as opaque yellow colonies. Lactose or sucrose-fermenting bacteria appear as yellow colonies.

### XPS Agar

**Composition** per liter:

Solution A .....	500.0mL
Solution B .....	500.0mL
pH 5.1 ± 0.2 at 25°C	

### Solution A:

**Composition** per 500mL:

Potatoes, infusion from .....	40.0g
Sucrose .....	15.0g
Peptone .....	5.0g
Glucose .....	4.0g
Casamino acids .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.79g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O solution .....	10.0mL

### Potatoes, Infusion From:

**Composition** per 500.0mL:

Potatoes .....	4.0g
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**Preparation of Potatoes, Infusion From:** Peel and dice potatoes. Add 400.0mL of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 30 min. Filter through cheesecloth.

### Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
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**Preparation of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O Solution:** Add Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Solution A:** Add components, except Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 490.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O solution. Mix thoroughly. Cool to 50°–55°C.

### Solution B:

**Composition** per 500mL:

Agar .....	20.0g
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**Preparation of Solution A:** Add agar to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C.

**Preparation of Medium:** Aseptically combine 500.0mL of solution A with 500.0mL of solution B. Mix thoroughly. Aseptically pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Xanthomonas campestris*.

### XPS Broth with Thymidine (Thymidine Auxotroph XPS Medium)

**Composition** per liter:

Potatoes, infusion from .....	40.0g
Sucrose .....	15.0g
Peptone .....	5.0g

Glucose .....	4.0g
Casamino acids .....	1.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.79g
Thymidine .....	10.0mg
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O solution .....	10.0mL
pH 5.1 ± 0.2 at 25°C	

### Potatoes, Infusion From:

**Composition** per 500.0mL:

Potatoes .....	4.0g
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**Preparation of Potatoes, Infusion From:** Peel and dice potatoes. Add 500.0mL of distilled/deionized water. Gently heat and bring to boiling. Continue boiling for 30 min. Filter through cheesecloth.

### Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O Solution:

**Composition** per 10.0mL:

Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O .....	0.5g
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**Preparation of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O Solution:** Add Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 10.0mL of sterile Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Xanthomonas oryzae*.

### Xylella Agar (LMG Medium 115)

**Composition** per liter:

Agar .....	17.0g
Yeast extract .....	10.0g
ACES .....	10.0g
Activated charcoal .....	2.0g
α-ketoglutarate .....	1.0g
KOH, 1N .....	40mL
Cysteine iron solution .....	20.0mL
pH 6.9 ± 0.2 at 25°C	

### Cysteine Iron Solution:

**Composition** per 20.0.0mL:

L-Cysteine·HCl .....	0.4g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> .....	0.25g

**Preparation of Cysteine Iron Solution:** Add L-cysteine·HCl and Fe<sub>4</sub>(P<sub>2</sub>O<sub>7</sub>)<sub>3</sub> to 20.0mL distilled/deionized water. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add ACES to 500.0mL distilled water at 50° C. Combine with a solution containing 40.0mL of 1 N KOH in 440.0mL distilled water. Add other components except cysteine iron solution. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 20.0mL sterile cysteine iron solution. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Xylella* spp.

### Xylella fastidiosa Medium (LMG 115)

**Composition** per liter:

Agar .....	17.0g
Yeast extract .....	10.0g
ACES buffer .....	10.0g
Activated charcoal .....	2.0g
L-Cysteine-iron solution .....	20.0mL
pH 6.9 ± 0.2 at 25°C	

**L-Cysteine-Iron Solution:****Composition per 20.0mL:**

L-Cysteine-HCl.....	0.4g
Fe <sub>4</sub> (P <sub>2</sub> O <sub>7</sub> ) <sub>3</sub> .....	0.25g

**Preparation of L-Cysteine-Iron Solution:** Add components to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add ACES to 500.0mL of distilled/deionized water at 50°C. Add a solution containing 40.0mL of 1N KOH in 440.0mL of distilled water. Mix thoroughly. Add the remaining components, except L-cysteine-iron solution. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 6.9 with KOH. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 20.0mL of sterile L-cysteine-iron solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation of *Xylella fastidiosa*.

***Xylophilus* Medium****Composition per liter:**

CaCO <sub>3</sub> .....	20.0g
Agar.....	15.0g
D-Galactose.....	10.0g
Yeast extract.....	10.0g
Ferric ammonium citrate solution.....	10.0mL

**Ferric Ammonium Citrate Solution:****Composition per 10.0mL:**

Ferric ammonium citrate.....	0.25g
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**Preparation of Ferric Ammonium Citrate Solution:** Add ferric ammonium citrate to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Add components, except ferric ammonium citrate solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°–55°C. Aseptically add 10.0mL of sterile ferric ammonium citrate solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and maintenance of *Xylophilus ampelina*.

**Xylose Sodium Deoxycholate Citrate Agar****Composition per liter:**

Agar.....	12.0g
Xylose.....	10.0g
Sodium citrate.....	5.0g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O.....	5.0g
Beef extract.....	5.0g
Peptone.....	5.0g
NaCl.....	2.5g
Sodium deoxycholate.....	2.5g
Ferric ammonium citrate.....	1.0g
Neutral Red (1% solution).....	2.5mL

pH 7.5 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling for 20 sec. Do not autoclave. Cool to 45°–50°C. Pour into sterile Petri dishes.

**Use:** For the cultivation of *Salmonella* species and some *Shigella* species.

**YA Halophile Medium****Composition per liter:**

NaCl.....	100.0g
Agar.....	15.0g
Sodium acetate·3H <sub>2</sub> O.....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	3.8g
KH <sub>2</sub> PO <sub>4</sub> .....	1.3g
Mg(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.0g
Yeast extract.....	1.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except magnesium nitrate, to tap water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add magnesium nitrate. Adjust pH 7.2 with sterile KOH. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of halophilic microorganisms, including *Bacillus halodenitrificans*.

**YB Medium****(Yeast Extract Beef Extract Medium)****Composition per liter:**

Agar.....	20.0g
Peptone.....	10.0g
Beef extract.....	7.0g
Yeast extract.....	5.0g
NaCl.....	3.0g
Thiourea.....	0.1g
Methanol.....	20.0mL

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components, except methanol, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add filter-sterilized methanol. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of bacteria that can utilize methanol as a carbon source, including *Achromobacter methanophila*, *Methanomonas methylovora*, *Methylobacterium* species, and *Pseudomonas methanolica*.

**YDC Medium****Composition per liter:**

CaCO <sub>3</sub> .....	20.0g
Glucose.....	20.0g
Agar.....	15.0g
Yeast extract.....	10.0g

pH 7.2 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Bdellovibrio* species.

**Yeast Agar, Van Niel's****Composition per liter:**

Agar.....	20.0g
Yeast extract.....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5g

pH 7.0–7.2 at 25°C

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and

bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a variety of microorganisms, including *Cytophaga* species, *Heliobacterium chlorum*, *Rhodomicrobium vannielii*, *Lysobacter enzymogenes*, *Rhodobacter* species, *Rhodocyclus gelatinosus*, *Rhodopseudomonas palustris*, and *Rhodospirillum rubrum*.

#### Yeast Agar, Van Niel's with Glutamate

**Composition per liter:**

Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
Monosodium glutamate .....	0.85g
pH 7.0–7.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of a variety of bacteria, including *Bacillus firmus*, *Cytophaga johnsonae*, *Heliobacterium chlorum*, *Lysobacter enzymogenes*, *Rhodobacter capsulatus*, *Rhodomicrobium vannielii*, *Rhodobacter sphaeroides*, *Rhodocyclus gelatinosus*, *Rhodocyclus gelatinosus*, *Rhodopseudomonas palustris*, and *Rhodospirillum rubrum*.

#### Yeast Agar, Van Niel's with 2.5% NaCl (ATCC Medium 1370)

**Composition per liter:**

NaCl .....	25.0g
Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
pH 7.0–7.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Chromatium vinosum* and *Rhodopseudomonas* species.

#### Yeast Agar, Van Niel's with 25% NaCl

**Composition per liter:**

NaCl .....	250.0g
Agar .....	20.0g
Yeast extract .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g
pH 7.0–7.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of halophilic bacteria, including *Haloarcula vallismortis*, *Halococcus morrhuae*, and *Halobacterium* species.

#### Yeast Agar, Van Niel's with Succinate

**Composition per liter:**

Agar .....	20.0g
Yeast extract .....	10.0g
Sodium succinate .....	1.35g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
pH 7.0–7.2 at 25°C	

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Rhodobacter capsulatus*.

#### Yeast Extract Agar

**Composition per liter:**

Agar .....	15.0g
Peptone .....	5.0g
Yeast extract .....	3.0g
pH 7.2 ± 0.2 at 25°C	

**Source:** This medium is available as a premixed powder from Oxoid Unipath.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the enumeration of microorganisms in potable and freshwater samples.

#### Yeast Extract Agar

**Composition per liter:**

Agar .....	20.0g
Yeast extract .....	1.0g
Buffer solution .....	2.0mL
pH 6.0 ± 0.2 at 25°C	

**Buffer Solution:**

**Composition per 400.0mL:**

KH <sub>2</sub> PO <sub>4</sub> .....	60.0g
Na <sub>2</sub> HPO <sub>4</sub> .....	40.0g

**Preparation of Buffer Solution:** Add 40.0g of Na<sub>2</sub>HPO<sub>4</sub> to 300.0mL of distilled/deionized water. Mix thoroughly. Add 60.0g of KH<sub>2</sub>PO<sub>4</sub>. Mix thoroughly. Adjust pH to 6.0.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes.

**Use:** For the identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*.

#### Yeast Extract Glucose Calcium Carbonate Agar

**Composition per liter:**

CaCO <sub>3</sub> .....	20.0g
Glucose .....	20.0g
Agar .....	15.0g
Yeast extract .....	10.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or



flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the isolation and cultivation of *Erwinia* species.

### Yeast Extract Glucose Medium

**Composition** per liter:

Agar .....	15.0g
Yeast extract .....	10.0g
Glucose .....	10.0g

**Preparation of Medium:** Add components to tap water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of a variety of bacteria, including *Streptomyces* species, *Rhodococcus* species, and others.

### Yeast Extract Mannitol Agar

**Composition** per liter:

Agar .....	15.0g
Mannitol .....	10.0g
CaCO <sub>3</sub> .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Yeast extract .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g

pH 6.8–7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Omit CaCO<sub>3</sub> if a clear solution is needed. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of members of the Rhizobiaceae.

### Yeast Extract Medium

**Composition** per liter:

Yeast extract .....	10.0g
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**Preparation of Medium:** Add yeast extract to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas cepacia*.

### Yeast Extract Medium with Sodium Sulfide

**Composition** per liter:

Yeast extract .....	10.0g
Na <sub>2</sub> S .....	0.15g

**Preparation of Medium:** Add yeast extract to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Immediately prior to inoculation, add 0.15g of Na<sub>2</sub>S. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Rhodospirillum molischianum*.

### Yeast Extract Mineral Agar

**Composition** per liter:

Agar .....	15.0g
Yeast extract .....	4.0g
NaHPO <sub>4</sub> ·12H <sub>2</sub> O .....	3.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g

NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.03g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Bacillus azotoformans*.

### Yeast Extract Mineral Medium (DSMZ Medium 259)

**Composition** per liter:

Agar .....	15.0g
Yeast extract .....	4.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	3.5g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
NH <sub>4</sub> Cl .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.03g

pH 7.1 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bacillus azotoformans*.

### Yeast Glucose Broth (YGB)

**Composition** per liter:

Beef extract .....	10.0g
Peptone .....	10.0g
NaCl .....	5.0g
Glucose .....	5.0g
Yeast extract .....	3.0g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Enterococcus faecalis*, *Streptococcus agalactiae*, and *Rhodobacter sphaeroides*.

### Yeast Malate Medium

**Composition** per liter:

Yeast extract .....	5.0g
Sodium malate .....	1.0g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodopseudomonas viridis*.

### Yeast Malate Medium

**Composition** per liter:

KH <sub>2</sub> PO <sub>4</sub> ·12H <sub>2</sub> O .....	1.0g
NaHCO <sub>3</sub> .....	1.0g
Sodium malate .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
Trace elements solution .....	1.0mL

pH 6.8 ± 0.2 at 25°C

**Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except trace elements solution, to distilled/deionized water and bring volume to 999.0mL. Mix thoroughly. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically add 1.0mL of sterile trace elements solution. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Rhodopseudomonas viridis*.

**Yeast Malate Medium  
(LMG 176)**

**Composition per 1001.0mL:**

KH <sub>2</sub> PO <sub>4</sub> ·12H <sub>2</sub> O .....	1.0g
NaHCO <sub>3</sub> .....	1.0g
Sodium malate .....	1.0g
Yeast extract .....	1.0g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5g
Trace elements solution .....	1.0mL

pH 6.8–7.0 at 25°C

**Trace Elements Solution:****Composition per liter:**

H <sub>3</sub> BO <sub>3</sub> .....	0.3g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.2g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	0.03g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.03g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.02g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.01g

**Preparation of Trace Elements Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 6.8–7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodopseudomonas viridis*.

**Yeast Mannitol Agar**

**Composition per liter:**

Agar .....	15.0g
Mannitol .....	10.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Yeast extract .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Rhizobium* and *Azorhizobium* species.

**Yeast Mannitol Agar**

**Composition per liter:**

Agar .....	20.0g
Mannitol .....	10.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	1.2g
KH <sub>2</sub> PO <sub>4</sub> .....	0.55g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
NaCl .....	0.25g
Yeast extract .....	0.25g
CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	30.0mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.5mg
H <sub>3</sub> BO <sub>3</sub> .....	500.0µg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	400.0µg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	160.0µg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	80.0µg

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Bradyrhizobium japonicum* and *Rhizobium leguminosarum*.

**Yeast Mannitol Agar, Modified**

**Composition per liter:**

Agar .....	15.0g
Mannitol .....	10.0g
CaCO <sub>3</sub> .....	4.0g
K <sub>2</sub> HPO <sub>4</sub> .....	0.5g
Yeast extract .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Amycolata autotrophica*, *Amycolatopsis orientalis*, *Nocardioideis albus*, *Promicromonospora citrea*, *Rhizobium fredii*, *Rhizobium galegae*, *Rhizobium leguminosarum*, *Rhizobium loti*, *Rhizobium meliloti*, and *Rhizobium tropici*.

**Yeast Nitrogen Base Glucose Broth**

**Composition per liter:**

Yeast nitrogen base .....	25.0mL
Glucose solution .....	25.0mL

pH 5.6 ± 0.2 at 25°C

**Yeast Nitrogen Base:****Composition per 500.0mL:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	5.0g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.5g
NaCl .....	0.1g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.1g
DL-Methionine .....	0.02g
DL-Tryptophan .....	0.02g
L-Histidine·HCl .....	0.01g
Inositol .....	2.0mg
H <sub>3</sub> BO <sub>3</sub> .....	0.5mg

ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.4mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	0.4mg
Thiamine-HCl .....	0.4mg
Pyroxidine-HCl .....	0.4mg
Niacin.....	0.4mg
Calcium pantothenate .....	0.4mg
<i>p</i> -Aminobenzoic acid.....	0.2mg
Riboflavin .....	0.2mg
FeCl <sub>3</sub> .....	0.2mg
Na <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O .....	0.2mg
KI .....	0.1mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.04mg
Folic acid.....	2.0µg
Biotin .....	2.0µg

**Source:** Yeast nitrogen base is available as a premixed powder from BD Diagnostic Systems.

**Preparation of Yeast Nitrogen Base:** Add components to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Filter sterilize.

#### Glucose Solution:

**Composition per 500.0mL:**

Glucose .....	10.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 500.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Aseptically combine 25.0mL of sterile yeast nitrogen base and 25.0mL of sterile glucose solution. Mix thoroughly.

**Use:** For the cultivation and enrichment of yeast from sewage and polluted waters.

### Yeast Peptone Agar (ATCC 1858)

**Composition per liter:**

Agar .....	15.0g
Yeast extract.....	2.5g
Peptone.....	2.5g

pH 7.0 ± 0.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Cytophaga lytica*, *Pseudomonas lanceolata*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rhodopseudomonas blastica*, *Rhodopseudomonas palustris*, *Rhodospirillum rubrum*, *Rubrivivax gelatinosus*, *Sphaerotilus natans*, and *Zoogloea ramigera*.

### Yeast Peptone Broth

**Composition per liter:**

Yeast extract.....	2.5g
Peptone.....	2.5g

pH 7.0 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Rhodopseudomonas* species.

### Yeast Peptone Salt Medium

**Composition per liter:**

Agar .....	15.0g
Peptone .....	2.5g
Yeast extract .....	2.5g
NaCl .....	1.25g

pH 7.0 ± 0.4 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Deinobacter grandis*.

### Yeast Water Agar

**Composition per liter:**

Glucose.....	20.0g
Agar .....	15.0g
Casein hydrolysate .....	5.0g
Yeast extract .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.55g
KCl .....	0.4g
CaCl <sub>2</sub> .....	0.13g
MgCl <sub>2</sub> ·7H <sub>2</sub> O.....	0.13g
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	2.5mg
MnSO <sub>4</sub> ·4H <sub>2</sub> O.....	2.5mg
Bromcresol Green solution.....	1.0mL

#### Bromcresol Green Solution:

**Composition per 10.0mL:**

Bromcresol Green .....	0.22g
Ethanol .....	10.0mL

**Preparation of Bromcresol Green Solution:** Add Bromcresol Green to 10.0mL of ethanol. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Zymomonas* species.

### Yeastrel Agar

**Composition per liter:**

Agar .....	15.0g
Peptone .....	9.5g
Yeastrel .....	7.0g
Lab-Lemco (meat extract) .....	5.0g
NaCl .....	5.0g

pH 7.0 ± 0.2 at 25°C

**Source:** Lab-lemco is available from Oxoid Unipath. Yeastrel is produced by Mapleton's Foods Ltd., Moss Street, Liverpool and is available from health food shops.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Aeromonas salmonicida*.

### YEPG Medium

**Composition per liter:**

Glucose.....	1.0g
Polypeptone .....	2.0g

NH <sub>4</sub> NO <sub>3</sub> .....	0.2g
Yeast extract .....	0.2g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.0. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Pseudomonas putida* and *Pseudomonas fluorescens*.

#### YEPP Medium

##### (Yeast Extract Proteose Peptone Medium)

**Composition** per liter:

Agar .....	15.0g
Proteose peptone .....	10.0g
NaCl .....	5.0g
Yeast extract .....	3.0g
pH 7.2-7.4 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Pseudomonas* species.

#### YGC Medium

##### (Yeast Extract Glucose Carbonate Medium) (ATCC Medium 73)

**Composition** per liter:

Agar .....	20.0g
Glucose .....	20.0g
CaCO <sub>3</sub> .....	20.0g
Yeast extract .....	10.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 30 min at 10 psi pressure–115°C. Cool to 48°C. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Xanthomonas* species, *Erwinia* species, *Kluyvera* species, *Rhodococcus* species, *Streptomyces* species, *Pseudomonas pseudoalcaligenes*, and *Xylophilus ampelinus*.

#### YGC Medium with Glutamic Acid

##### (Yeast Extract Glucose Carbonate Medium with Glutamic Acid)

**Composition** per liter:

Agar .....	20.0g
Glucose .....	20.0g
CaCO <sub>3</sub> .....	20.0g
Agar .....	20.0g
Yeast extract .....	10.0g
Glutamic acid .....	0.1g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 30 min at 10 psi pressure–115°C. Cool to 48°C. Mix thoroughly. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas campestris*.

#### YGCB Salt Medium

**Composition** per liter:

NaCl .....	50.0g
Beef broth .....	10.0g
Glucose .....	10.0g
Peptone .....	10.0g
Triammonium citrate .....	5.0g
Yeast extract .....	5.0g
Sodium acetate .....	2.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	50.0mg
Tween 80 .....	1.0mL
pH 6.7 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Tetragenococcus halophila*.

#### YGCP Medium

##### (Yeast Extract Glucose Carbonate Peptone Medium)

**Composition** per liter:

Glucose .....	20.0g
Agar .....	17.5g
CaCO <sub>3</sub> .....	10.0g
Yeast extract .....	2.5g
Peptone .....	2.5g
NaCl .....	1.0g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
MgSO <sub>4</sub> .....	0.5g

**Preparation of Medium:** Add components, except calcium carbonate, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Adjust pH to 7.2. Add calcium carbonate. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Xanthomonas campestris* and *Xanthomonas oryzae*.

#### YI-S Medium

**Composition** per liter:

YI broth .....	880.0mL
Bovine serum, heat inactivated .....	100.0mL
Vitamin mixture 18 .....	20.0mL

**Source:** Vitamin mixture 18 is available from Bio-fluids, Inc., Rockville, MD.

#### YI Broth:

**Composition** per liter:

YI base stock .....	780.0mL
10× Glucose buffer stock .....	100.0mL

#### YI Base Stock:

**Composition** per 780.0mL:

Yeast extract .....	30.0g
L-Cysteine·HCl .....	1.0g
NaCl .....	1.0g
Ascorbic acid .....	0.2g
Ferric ammonium citrate .....	228.0mg

#### 10× Glucose Buffer Stock:

**Composition** per 100.0.0mL:

Glucose .....	10.0g
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K <sub>2</sub> HPO <sub>4</sub> .....	1.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.6g

**Preparation of 10× Glucose Buffer Stock:** Add components to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Filter-sterilize.

**Preparation of YI Base Stock:** Add components to 600.0mL of distilled/deionized water. Mix thoroughly. Bring volume to 780.0mL with distilled/deionized water. Adjust pH to 6.8 with 1N NaOH. Distribute in 78.0mL aliquots to 100.0mL screw-capped bottles. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Preparation of YI Broth:** Aseptically add 10.0mL of 10× glucose buffer stock to 78.0mL of cooled YI base stock. Adjust osmolarity with NaCl to 380.0milliosmols/kg

**Preparation of Medium:** Aseptically add 2.0mL of vitamin mixture 18 and 10.0mL of heat-inactivated bovine serum to 88.0mL of YI broth. Distribute in 13.0mL aliquots to 16 x 125mm screw-capped test tubes. Store at 4°C in the dark with the caps screwed on tightly. Use within 96 hr.

**Use:** For the cultivation of *Entamoeba* species.

### YMA Agar

**Composition per liter:**

Agar .....	15.0g
Mannitol .....	10.0g
CaCO <sub>3</sub> .....	4.0g
KH <sub>2</sub> PO <sub>4</sub> .....	0.5g
Yeast extract .....	0.4g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.2g
NaCl .....	0.1g

pH 6.8 ± 0.2 at 25°C

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Rhizobium fredii*, *Rhizobium gallegae*, *Rhizobium huakuii*, *Rhizobium leguminosarum*, *Rhizobium loti*, *Rhizobium meliloti*, *Rhizobium phaseoli*, and *Rhizobium trifolii*.

### Yopp's Medium

**Composition per liter:**

NaCl .....	116.88g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	10.68g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	10.0g
KCl .....	2.0g
CaNO <sub>3</sub> ·4H <sub>2</sub> O .....	1.0g
Glycyl-glycine buffer .....	0.5g
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O .....	0.065g
Ferric EDTA .....	5.0mg
Trace metal solution .....	1.0mL

pH 7.8 ± 0.2 at 25°C

### Trace Metal Solution:

**Composition per liter:**

MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	2.0g
H <sub>3</sub> BO <sub>3</sub> .....	0.5g
Zn(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.5g
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O .....	0.025g
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.025g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.025g
VOSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.025g
HCl .....	3.0mL

**Preparation of Trace Metal Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the isolation and cultivation of halophilic cyanobacteria.

### YP87 Medium

**Composition per liter:**

Na <sub>2</sub> SO <sub>4</sub> .....	4.0g
NaHCO <sub>3</sub> .....	1.3g
KCl .....	0.5g
Yeast extract .....	0.5g
MgCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.4g
NH <sub>4</sub> Cl .....	0.25g
L-Ascorbic acid .....	0.2g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.2g
Sodium thioglycolate .....	0.2g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.15g
Resazurin .....	1.0mg
Modified Wolfe's mineral solution .....	10.0mL
Wolfe's vitamin solution .....	10.0mL
Sodium lactate, 60% syrup .....	3.0mL

pH 7.5 ± 0.2 at 25°C

### Modified Wolfe's Mineral Solution:

**Composition per liter:**

MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	3.0g
Nitrilotriacetic acid .....	1.5g
NaCl .....	1.0g
MnSO <sub>4</sub> ·H <sub>2</sub> O .....	0.5g
CaCl <sub>2</sub> .....	0.1g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.1g
FeSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.1g
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O .....	0.01g
CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.01g
H <sub>3</sub> BO <sub>3</sub> .....	0.01g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.01g
NaWO <sub>4</sub> ·2H <sub>2</sub> O .....	0.01g
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	0.01g

**Preparation of Modified Wolfe's Mineral Solution:** Add nitrilotriacetic acid to 500.0mL of distilled/deionized water. Adjust pH to 6.5 with KOH. Add remaining components one at a time. Add distilled/deionized water to 1.0L. Adjust pH to 6.8.

### Wolfe's Vitamin Solution:

**Composition per liter:**

Pyridoxine-HCl .....	10.0mg
p-Aminobenzoic acid .....	5.0mg
Lipoic acid .....	5.0mg
Nicotinic acid .....	5.0mg
Riboflavin .....	5.0mg
Thiamine-HCl .....	5.0mg
Calcium DL-pantothenate .....	5.0mg
Biotin .....	2.0mg
Folic acid .....	2.0mg
Vitamin B <sub>12</sub> .....	0.1mg

**Preparation of Wolfe's Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except L-ascorbic acid, NaHCO<sub>3</sub>, and sodium thioglycolate, to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.5. Gently heat and bring to boiling. Cool while sparging with 100% N<sub>2</sub>. Add L-ascorbic acid, NaHCO<sub>3</sub>, and sodium thioglycolate. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Distribute into tubes or bottles. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Thermodesulfovibrio yellowstonii*.

**YPSC Agar  
(Yeast Extract Peptone  
Sulfate Cysteine Agar)**

**Composition per liter:**

Agar .....	15.0g
Yeast extract .....	1.0g
Peptone .....	1.0g
Sodium acetate·3H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.05g
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 7.5 with sterile 10M NaOH. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Bdellovibrio* species.

**YPSC Agar, Cation-Supplemented**

**Composition per liter:**

Sodium acetate·3H <sub>2</sub> O .....	50.0g
Agar .....	15.0g
Peptone .....	10.0g
Yeast extract .....	10.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.74g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.29g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.05g
Bacitracin solution .....	10.0mL

**Bacitracin Solution:**

**Composition per 10.0mL:**

Bacitracin .....	6,000U
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**Preparation of Bacitracin Solution:** Add bacitracin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Preparation of Medium:** Add components, except bacitracin solution, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 45°–50°C. Aseptically add sterile bacitracin solution. Mix thoroughly. Pour into sterile Petri dishes or distribute into sterile tubes.

**Use:** For the cultivation and enumeration of *Bdellovibrio* species.

**YPSC Medium  
(Yeast Extract Peptone  
Sulfate Cysteine Medium)**

**Composition per liter:**

Yeast extract .....	1.0g
Peptone .....	1.0g

Sodium acetate·3H <sub>2</sub> O .....	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.25g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.25g
L-Cysteine·HCl·H <sub>2</sub> O .....	0.05g
pH 7.5 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Adjust pH to 7.5 with sterile 10M NaOH.

**Use:** For the cultivation and maintenance of *Bdellovibrio* species.

**YSP Agar**

**Composition per liter:**

Sucrose .....	20.0g
Agar .....	12.0g
Peptone .....	10.0g
Yeast extract .....	5.0g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation of *Xanthomonas albilineans*.

**YTG Medium**

**Composition per liter:**

Tryptone .....	10.0g
Na <sub>2</sub> CO <sub>3</sub> .....	5.3g
Yeast extract .....	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O .....	0.356g
L-Cysteine·HCl .....	0.2g
Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.2g
KCl .....	0.075g
Resazurin .....	1.0mg
Glucose solution .....	20.0mL
pH 10.1 ± 0.2 at 25°C	

**Glucose Solution:**

**Composition per 20.0mL:**

D-Glucose .....	3.0g
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**Preparation of Glucose Solution:** Add glucose to distilled/deionized water and bring volume to 20.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Preparation of Medium:** Prepare and dispense medium under 100% N<sub>2</sub>. Add components, except glucose solution, to distilled/deionized water and bring volume to 980.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub> for 30 min. Anaerobically distribute 9.8mL volumes into tubes. Autoclave for 15 min at 15 psi pressure–121°C. Aseptically and anaerobically add 0.2mL of sterile glucose solution to each tube. Adjust pH to 10.1 with sterile anaerobic 3N NaOH solution.

**Use:** For the cultivation of *Clostridium paradoxum* and *Clostridium thermoacetaliphilum*.

**YTSS Medium, Half-Strength  
(DSMZ Medium 974)**

**Composition per liter:**

Sea salts .....	20.0g
Yeast extract .....	2.0g
Tryptone .....	1.25g
pH 7.0 ± 0.2 at 25°C	

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Roseovarius nubinhibens*, a budding bacterium from a hypersaline lake, and *Silicibacter pomeroyi*, a marine bacterium.

### Z Medium

**Composition per liter:**

Casein hydrolysate .....	10.0g
NaCl .....	10.0g
Yeast extract .....	5.0g
Glucose .....	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.367g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C.

**Use:** For the cultivation of *Alcaligenes eutrophus* (*Ralsonia eutropha*), a hydrogen-producing phytopathogen.

### ZF2 Medium (DSMZ Medium 943)

**Composition per liter:**

NaHCO <sub>3</sub> .....	3.8g
Yeast extract .....	3.0g
(NH <sub>4</sub> )HCO <sub>3</sub> .....	0.45g
MgSO <sub>4</sub> ·6H <sub>2</sub> O .....	0.13g
CaCl <sub>2</sub> ·2H <sub>2</sub> O .....	0.12g
Resazurin .....	0.5mg
Phosphate buffer .....	10.0mL
Glycine solution .....	5.0mL
Arginine solution .....	5.0mL
Na <sub>2</sub> S·9H <sub>2</sub> O solution .....	5.0mL
Dithionite solution .....	1.0mL
Seven vitamin solution .....	1.0mL
Trace elements solution SL-10 .....	1.0mL
Selenite-tungstate solution .....	1.0mL

pH 7.3 ± 0.2 at 25°C

**Arginine Solution:**

**Composition per 10.0mL:**

Arginine-HCl .....	3.5g
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**Preparation of Arginine Solution:** Add arginine-HCl to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize.

**Glycine Solution:**

**Composition per 100.0mL:**

Glycine .....	15.0g
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**Preparation of Glycine Solution:** Add glycine to distilled/deionized water and bring volume to 100.0mL. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Na<sub>2</sub>S·9H<sub>2</sub>O Solution:**

**Composition per 10mL:**

Na <sub>2</sub> S·9H <sub>2</sub> O .....	0.3g
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**Preparation of Na<sub>2</sub>S·9H<sub>2</sub>O Solution:** Add Na<sub>2</sub>S·9H<sub>2</sub>O to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to room temperature.

**Dithionite Solution:**

**Composition per 10mL:**

Na <sub>2</sub> -dithionite .....	0.25g
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**Preparation of Dithionite Solution:** Add Na<sub>2</sub>-dithionite to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Autoclave under 100% N<sub>2</sub> for 15 min at 15 psi pressure–121°C. Cool to 25°C.

**Seven Vitamin Solution:**

**Composition per liter:**

Pyridoxine hydrochloride .....	300.0mg
Thiamine-HCl·2H <sub>2</sub> O .....	200.0mg
Nicotinic acid .....	200.0mg
Vitamin B <sub>12</sub> .....	100.0mg
Calcium pantothenate .....	100.0mg
p-Aminobenzoic acid .....	80.0mg
D(+)-Biotin .....	20.0mg

**Preparation of Seven Vitamin Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Sparge with 100% N<sub>2</sub>. Mix thoroughly. Filter sterilize.

**Phosphate Buffer:**

**Composition per liter:**

Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O .....	43.0g
KH <sub>2</sub> PO <sub>4</sub> .....	5.44g

**Preparation of Phosphate Buffer:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.3. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C.

**Trace Elements Solution SL-10:**

**Composition per liter:**

FeCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.5g
CoCl <sub>2</sub> ·6H <sub>2</sub> O .....	190.0mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	100.0mg
ZnCl <sub>2</sub> .....	70.0mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O .....	36.0mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O .....	24.0mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O .....	2.0mg
HCl (25% solution) .....	10.0mL

**Preparation of Trace Elements Solution SL-10:**

Add FeCl<sub>2</sub>·4H<sub>2</sub>O to 10.0mL of HCl solution. Mix thoroughly. Add distilled/deionized water and bring volume to 1.0L. Add remaining components. Mix thoroughly. Sparge with 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Autoclave for 15 min at 15 psi pressure–121°C.

**Selenite-Tungstate Solution:**

**Composition per liter:**

NaOH .....	0.5g
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O .....	4.0mg
Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O .....	3.0mg

**Preparation of Selenite-Tungstate Solution:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Sparge with 100% N<sub>2</sub>. Filter sterilize.

**Preparation of Medium:** Prepare and dispense medium under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Add components, except phosphate buffer, glycine solution, arginine solution, dithionite solution, seven vitamin solution, and Na<sub>2</sub>S·9H<sub>2</sub>O solution, to distilled/deionized water and bring volume to 973.0mL. Mix thoroughly. Equilibrate with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to reach pH 7.3. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 25°C. Aseptically and anaerobically add 10.0mL phosphate buffer, 5.0mL glycine solution, 5.0mL arginine solution, 1.0mL dithionite solution, 1.0mL seven vitamin solution, and 5.0mL Na<sub>2</sub>S·9H<sub>2</sub>O solution. Mix thor-

oughly. Aseptically and anaerobically distribute into sterile tubes or flasks.

**Use:** For the cultivation of *Sedimentibacter saalensis* from freshwater sediment.

### ***Zoogloea* Medium**

**Composition** per liter:

Agar .....	15.0g
Pancreatic digest of casein .....	5.0g
Glycerol .....	5.0g
Yeast autolysate.....	1.0g
Sodium lactate.....	0.5g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into screw capped test tubes. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Zoogloea ramigera* and other *Zoogloea* species.

### ***Zymomonas* Agar**

**Composition** per liter:

Glucose.....	20.0g
Agar.....	15.0g
Yeast extract .....	5.0g

**Preparation of Medium:** Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

**Use:** For the cultivation and maintenance of *Zymomonas mobilis* and other *Zymomonas* species.





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