

## Nutrient Media

When unsealing an ampoule with a culture obtained from the All-Russian Collection of Microorganisms (VKM) and for initial reinoculations, it is recommended to use the medium specified for the strain in the Catalogue.

General comments to the descriptions of the media

1. In all cases when the composition of a medium is given as the description of particular solutions, this implies that the given solutions should be sterilized separately and mixed upon sterilization.
2. In all cases, besides when specified otherwise, a given mode of sterilization assumes autoclaving. If the sterilization conditions of a medium are not specified, they can be set arbitrarily within the limits accepted in the general microbiological practice.
3. The purity of the reagents is not specified in the descriptions. It is assumed that the respective reagents will be used, specifically, peptone, tryptone, yeast extract, etc. marked 'Bacto' or 'For microbiological work'. For some media, descriptions for preparing the components are given instead of using commercial concentrates.
4. The pH values given indicate the magnitudes, which a medium shall have prior to inoculation. In the cases when some solutions are described and errors are possible, it is specified: pH of the medium. An insufficiently alkaline medium shall be alkalized, usually with a sterile solution of NaOH or NaHCO<sub>3</sub>; an insufficiently acidic one should be acidified, usually with a sterile solution of HCl.

### 1. *ALLOMONAS ENTERICA* MEDIUM

Peptone 10.0 g

NaCl 20.0 g

Beef extract 5.0 g\*

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min.

\*Beef extract may be replaced with 3.0 g yeast extract.

### 2. YEAST WATER

Pressed yeast 200.0 g

Tap water 1000.0 ml

*Preparation of yeast water:* suspend 200.0 g of pressed yeast in 1000.0 ml of tap water and boil for 30 min. Twice filter hot through a paper filter or centrifuge.

Autoclave at 121°C for 15 min.

### 3. POTATO AGAR

Potato 200.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil 200.0 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, fill up distilled water to 1000.0 ml, add agar. Do not use new potatoes.

pH 7.0

Autoclave at 121°C for 15 min.

### 4. WORT AGAR

Wort extract (malt extract) 20.0 g

Agar 20.0 g

Distilled water 1000.0 ml

Autoclave at 121°C for 15 min.

### 5. PEPTONE MEAT AGAR

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

#### **6. PEPTONE MEAT BROTH**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

#### **7. OATMEAL AGAR (OA)**

Oatmeal 30.0 g  
Agar 15.0 g  
Tap water 1000.0 ml  
Cook 30.0 g of oatmeal in 1000.0 ml tap water for 20 min, filter through 2 layers of gauze, dilute to 1000.0 ml and add 15.0 g agar.  
Autoclave at 121°C for 15 min.

#### **8. PEPTONE MAIZE AGAR**

Peptone 5.0 g  
Maize extract 5.0 g  
Starch (soluble) 10.0 g  
NaCl 5.0 g  
CaCl<sub>2</sub> 0.5 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

#### **9. MALT AGAR 7 BALLING (MA7B)**

Malt extract Balling 7 degrees 1000.0 ml  
Agar 20.0 g  
Adjust pH to 7.0  
Autoclave at 111°C for 30 min.

#### **10. MALT AGAR 2 BALLING (MA2B)**

Malt extract Balling 2 degrees 1000.0 ml  
Agar 20.0 g  
Autoclave at 111°C for 30 min.

#### **11. MALT AGAR 3.5 BALLING (MA3.5B)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Agar 20.0 g  
Autoclave at 111°C for 30 min.

#### **12. CZAPEK MEDIUM (CZ)**

NaNO<sub>3</sub> 3.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g

KCl 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
Sucrose 30.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 6.0  
Autoclave at 121°C for 30 min.

### **13. POTATO-GLUCOSE AGAR (PDA)**

Grated potato 200.0 g  
Glucose 20.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.  
pH 6.5-7.0  
Autoclave at 121°C for 15 min.

### **14. POTATO-CARROT AGAR (PCA)**

Grated potato 20.0 g  
Grated carrot 20.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
Boil potato and carrot in 1000.0 ml of water for 1 h, filter cold through a cotton-gauze filter, add water to the initial volume and add agar. Do not use new potatoes.  
pH 7.0-7.1  
Autoclave at 121°C for 15 min.

### **15. LB MEDIUM**

Tryptone 10.0 g  
Yeast extract 5.0 g  
NaCl 10.0 g  
Tap water 1000.0 ml  
pH 7.5  
Autoclave at 121°C for 15 min.

### **16. YT MEDIUM**

Tryptone 8.0 g  
Yeast extract 5.0 g  
NaCl 5.0 g  
Tap water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

### **17. GLUCOSE PEPTONE AGAR (GPA)**

Glucose 40.0 g  
Peptone 10.0 g  
Yeast extract 5.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 111°C for 30 min.

### **18. GLUCOSE PEPTONE AGAR WITH 5% NaCl**

Glucose 40.0 g  
Peptone 10.0 g  
Yeast extract 5.0 g  
NaCl 50.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
Autoclave at 111°C for 30 min.

### **19. MALT AGAR 7 BALLING WITH 5% NaCl (MA7B+5% NaCl)**

Malt extract Balling 7 degrees 1000.0 ml  
NaCl 50.0 g  
Agar 20.0 g  
Autoclave at 111°C for 30 min.

### **20. LIESKE MEDIUM**

Mn-acetate 0.1 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
Autoclave at 111°C for 30 min.

### **21. MANNITOL AGAR WITH YEAST WATER**

Mannitol 10.0 g  
Agar 15.0 g  
Yeast water 100.0 ml  
Tap water 900.0 ml  
*Preparation of yeast water:* suspend 200.0 g of pressed yeast in 1000.0 ml of tap water. Twice filter hot through a paper filter or centrifuge.  
Autoclave at 121°C for 15 min.

### **22. MALT AGAR 3.5 BALLING WITH 60% SUCROSE (MA60S)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Sucrose 600.0 g  
Agar 20.0 g  
Autoclave at 121°C for 15 min.

### **23. MALT AGAR 3.5 BALLING WITH 40% SUCROSE (MA40S)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Sucrose 400.0 g  
Agar 20.0 g  
Autoclave at 121°C for 15 min.

### **24. MALT AGAR 3.5 BALLING WITH 20% SUCROSE (MA20S)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Sucrose 200.0 g  
Agar 20.0 g  
Autoclave at 121°C for 15 min.

### **25. MALT AGAR 3.5 BALLING WITH FILTER PAPER**

Malt extract Balling 3.5 degrees 1000.0 ml  
Agar 20.0 g  
Autoclave at 121°C for 15 min.  
Sterilize filter paper strips with dry heat and soak it with sterile medium.

### **26. MANURE AGAR**

Horse manure 100-125 g

Agar 25.0 g

Distilled water 1000.0 ml

pH 6.5-7.0

Boil manure in 1000.0 ml of water for 10 min, then keep for 16-20 hours, filter through 1-2 layers of filter paper, adjust to the initial volume, add agar.

Autoclave at 121°C for 15 min.

### **27. PEPTONE LACTOSE AGAR**

Peptone 10.0 g

Lactose 10.0 g

Agar 15.0 g

Tap water 1000.0 ml

Autoclave at 121°C for 15 min.

### **28. TRYPTOSE AGAR**

Tryptose 20.0 g

Glucose 1.0 g

NaCl 5.0 g

Agar 15.0 g

Thiamine-HCl 0.05 g

Distilled water 1000.0 ml

Autoclave at 121°C for 15 min.

### **29. PEA AGAR**

Yellow peas 100.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

Sucrose 10.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil peas in 1000.0 ml of water, filter through gauze, add water to the initial volume; add phosphate, sucrose and agar.

Autoclave at 121°C for 15 min.

### **30. AZOTOBACTER MEDIUM 1**

Glucose 5.0 g

Mannitol 5.0 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 5.0 mg

K<sub>2</sub>HPO<sub>4</sub> 0.9 g

KH<sub>2</sub>PO<sub>4</sub> 0.1 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g

CaCO<sub>3</sub> 5.0 g

Agar 15.0 g

Distilled water 950.0 ml

pH 7.3

Autoclave at 121°C for 15 min.

Sterilize glucose and mannitol separately (in 50.0 ml H<sub>2</sub>O) and add to the medium after autoclaving.

### **31. CABBAGE AGAR**

Cabbage 50.0 g

Glucose 20.0 g

Peptone 10.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil 50.0 g of cabbage in 1000.0 ml of water, filter cabbage and adjust the volume of broth to the initial value.

pH 7.0

Autoclave at 111°C for 30 min.

### **32. CURD DECOCTION**

Pour 9000.0 ml of distilled water into 3.0 kg of curd and add 150.0 of dry *Aspergillus terricola* mycelium, shake, pour chloroform, seal with a stopper. Decoct at 37°C for 10 days, adjust pH to 7.0 with 1 N NaOH. The decoction shall contain 400 mg% amine nitrogen and 300 mg% tryptophan.

### **33. PEPTONE MEAT AGAR WITH 0.2% UREA**

Peptone 10.0 g

NaCl 5.0 g

Beef extract 3.0 g

Agar 20.0 g

Distilled water 1000.0 ml

Do not adjust pH; pH raises to about 8 due to heat degraded urea.

Autoclave at 121°C for 15 min.

10.0 ml filter-sterilized 20% urea solution is added aseptically after autoclaving to 1000.0 ml cooled, molten, agar. The medium is then immediately dispensed aseptically.

### **34. SOIL EXTRACT**

Dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121°C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121°C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

### **35. NITROBACTER MEDIUM 1**

Solution 1 (see below) 0.5 ml

Solution 2 (see below) 0.5 ml

Solution 3 (see below) 1.0 ml

Solution 4 (see below) 0.5 ml

Solution 5 (see below) 0.5 ml

Solution 6 (see below) 0.1 ml

Distilled water to 1000.0 ml

*Solution 1:*

CaCl<sub>2</sub> 2.0 g

Distilled water 100.0 ml

*Solution 2:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g

Distilled water 100.0 ml

*Solution 3:*

Chelated iron 0.1 g

Distilled water 100.0 ml

*Solution 4:*

Na<sub>2</sub>MoO<sub>4</sub> 0.1 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml

*Solution 5:*

NaNO<sub>3</sub> 41.4 g  
Distilled water 100.0 ml

*Solution 6:*

K<sub>2</sub>HPO<sub>4</sub> 1.74 g  
Distilled water 100.0 ml

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

**36. PROPIONIBACTERIUM MEDIUM**

Yeast extract 10.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
Na<sub>2</sub>HPO<sub>4</sub> × 2 H<sub>2</sub>O 3.0 g  
Na-lactate (70%) 40.0 ml  
Distilled water 1000.0 ml  
Dissolve all ingredients and add Na-lactate.  
pH 7.0  
Autoclave at 121°C for 15 min.

**37. KNOP MEDIUM WITH FILTER PAPER**

Ca(NO<sub>3</sub>)<sub>2</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.25 g  
MgSO<sub>4</sub> 0.25 g  
FeCl<sub>3</sub> Traces  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 6.5-7.0  
Autoclave at 121°C for 15 min.  
Sterilize filter paper strips by dry heat and soak with sterile medium.

**38. MALT AGAR 7 BALLING WITH 12% NaCl (MA12NaCl)**

Malt extract Balling 7 degrees 1000.0 ml  
NaCl 120.0 g  
Agar 20.0 g  
Autoclave at 121°C for 15 min.

**39. MALT 7 BALLING AGAR WITH 1% NaCl**

Malt extract Balling 7 degrees 1000.0 ml  
NaCl 10.0 g  
Agar 20.0 g  
Autoclave at 121°C for 15 min.

**40. AZOTOBACTER MEDIUM 2**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
K<sub>2</sub>HPO<sub>4</sub> 0.8 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
CaSO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 g  
FeCl<sub>3</sub> Traces  
Na<sub>2</sub>MoO<sub>4</sub> Traces  
Yeast extract 0.5 g  
Sucrose 20.0 g  
Agar 15.0-20.0 g  
Distilled water 1000.0 ml

pH 7.2

Autoclave at 111°C for 30 min.

#### **41. FLAVOBACTERIUM MEDIUM**

Na-caseinate 2.0 g

Yeast extract 0.5 g

Peptone 1.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

Agar 12.0 g

Distilled water 1000.0 ml

pH 7.4

Autoclave at 121°C for 15 min.

#### **42. PSEUDOMONAS SACCHAROPHILA MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 4.4 g

Na<sub>2</sub>HPO<sub>4</sub> 4.8 g

NH<sub>4</sub>Cl 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

Agar (if needed) 20.0 g

Distilled water 985.0 ml

*Solution 2:*

Ferric ammonium citrate 50.0 mg

CaCl<sub>2</sub> 5.0 mg

Distilled water 5.0 ml

*Solution 3:*

Sucrose 1.0 g

Distilled water 10.0 ml

Autoclave solutions 1 and 2 separately at 121°C for 15 min, solution 3 at 111°C for 30 min and mix aseptically.

#### **43. SEA WATER MEDIUM WITH YEAST EXTRACT**

Sea salt 37.9 g

Yeast extract 3.0 g

Peptone 10.0 g

Agar 20.0 g

Distilled water to 1000.0 ml

pH 7.2-7.4

Autoclave at 121°C for 15 min.

#### **44. HALOBACTERIUM MEDIUM 1**

*Solution 1:*

NaCl 250.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 g

KCl 5.0 g

CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

Tap water 800.0 ml

*Solution 2:*

Yeast extract 10.0 g

Tryptone 2.5 g

Agar 20.0 g

Tap water 200.0 ml

Autoclave solutions separately at 121°C for 15 min and mix aseptically.



#### **45. STARVED AGAR**

Agar 20.0 g  
Distilled water 1000.0 ml  
Autoclave at 121°C for 15 min.

#### **46. SP MEDIUM FOR *STIGMATELLA AURANTIACA***

Raffinose 1.0 g  
Sucrose 1.0 g  
Galactose 1.0 g  
Starch (soluble) 5.0 g  
Casitone 2.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.25 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
Autoclave at 111°C for 30 min.

#### **47. CM + YE MEDIUM**

Casamino acids 7.5 g  
Yeast extract 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g  
Na-citrate 3.0 g  
KCl 2.0 g  
NaCl 200.0 g  
Agar 15.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O in 0.01N HCl (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Solution of FeSO<sub>4</sub> × 7 H<sub>2</sub>O:*  
HCl (0.01 N) 100.0 ml  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 4.98 g  
pH 7.4 (adjust with 1N NaOH)  
Autoclave solutions separately at 121°C for 15 min and mix aseptically.

#### **48. CASEIN MEDIUM**

NaCl 250.0 g  
Casein hydrolysate 5.0 g  
Yeast extract 5.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 g  
KCl 2.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.2 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.4 (adjust with 1N NaOH)  
Autoclave at 121°C for 15 min.

#### **49. HALOBACTERIUM MEDIUM 2**

*Solution 1:*  
NaCl 120.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 50.0 g  
K<sub>2</sub>SO<sub>4</sub> 5.0 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Distilled water 500.0 ml  
*Solution 2:*  
Tryptone 5.0 g

Yeast extract 5.0 g  
Agar 20.0 g  
Distilled water 500.0 ml  
pH 6.8  
Autoclave solutions separately at 121°C for 15 min and mix aseptically.

#### **50. YEAST GLUCOSE AGAR**

Yeast extract 5.0 g  
Peptone 5.0 g  
Glucose 10.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 111°C for 30 min.

#### **51. CASITONE AGAR**

Casitone 3.0 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  1.36 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 121°C for 15 min.

#### **52. CASITONE YEAST MEDIUM**

Casitone 3.0 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  1.36 g  
Yeast extract 1.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 121°C for 15 min.

#### **53. THERMUS THERMOPHILUS MEDIUM**

Yeast extract 4.0 g  
Polypeptone 8.0 g  
NaCl 2.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

#### **54. DESULFOVIBRIO MEDIUM WITH 1% NaCl**

$\text{K}_2\text{HPO}_4$  0.01 g  
NaCl 10.0 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.2 g  
Na-lactate (40%) 4.0 ml  
Solution of *More* salt (see below) 1.0 ml  
Yeast extract 1.0 g  
Ascorbic acid 0.1 g  
Agar 6.0 g  
Distilled water 1000.0 ml  
*Solution of More salt:*  
 $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$  1.0 g  
Distilled water 5.0 ml

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

### **55. CAULOBACTER MEDIUM**

Peptone 2.0 g

Yeast extract 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

Agar 10.0 g

Tap water 1000.0 ml

Autoclave at 121°C for 15 min.

### **56. CAULOBACTER MEDIUM WITH GLUCOSE**

Peptone 2.0 g

Yeast extract 1.0 g

Glucose 2.0 g

Riboflavin 1.0 mg

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

Agar 10.0 g

Tap water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min.

### **57. PEPTONE MEAT AGAR WITH 1% UREA**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Urea 10.0 g

Agar 20.0 g

Distilled water 1000.0 ml

Do not adjust pH; pH raises to about 8 due to heat degraded urea.

Autoclave at 121°C for 15 min.

### **58. HALOBACTERIUM MEDIUM 3**

*Solution 1:*

Casamino acids 7.5 g

Yeast extract 10.0 g

Na-citrate 3.0 g

KCl 2.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g

FeCl<sub>3</sub> traces

NaCl 250.0 g

Distilled water 750.0 ml

*Solution 2:*

Agar 25.0 g

Distilled water 250.0 ml

pH 7.4

Autoclave solutions separately at 121°C for 15 min. and mix aseptically.

### **59. PSEUDONOCARDIA THERMOPHILA MEDIUM**

Peptone 2.5 g

Meat extract 2.5 g

NaCl 2.5 g

Yeast extract 0.1 g

Glucose 2.5 g

Sucrose 5.0 g

Casein acidic hydrolysate 0.1 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 111°C for 30 min.

#### **60. ALFALFA AGAR**

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NaCl 0.2 g  
CaSO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> Traces  
Mannitol 20.0 g  
Alfalfa meal 10.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2.  
Autoclave at 111°C for 20 min.

#### **61. MEDIUM FOR NITROGEN-FIXING *SPIRILLUM***

K<sub>2</sub>HPO<sub>4</sub> 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.4 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
NaCl 0.1 g  
CaCl<sub>2</sub> 0.02 g  
FeCl<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.0 2 g  
Na-malate 5.0 g  
Yeast extract 50.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

#### **62. MANURE TINCTURE**

Cow manure (fresh) 1.0 kg  
Distilled water 3000.0 ml  
Boil, squeeze through gauze into a bottle and dilute to 3000.0 ml.

#### **63. MEDIUM WITH CURD DECOCTION**

Curd decoction 61.0 ml  
Manure tincture 184.0 ml  
Na-acetate 1.0 g  
Agar 20.0 g  
Tap water 735.0 ml  
pH 7.8

*Preparation of curd decoction:* pour 9000.0 ml of distilled water into 3.0 kg of curd and add 150.0 of dry *Aspergillus terricola* mycelium, shake, pour chloroform, seal with a stopper. Decoct at 37°C for 10 days, adjust pH to 7.0 with 1 N NaOH. The decoction shall contain 400 mg% amine nitrogen and 300 mg% tryptophan.

*Preparation of manure tincture:* mix cow manure (fresh) 1.0 kg and distilled water 3000.0 ml. Boil, squeeze through gauze into a bottle and dilute to 3000.0 ml.  
Autoclave at 121°C for 15 min.

#### **64. PEPTONE YEAST AGAR**

Peptone 5.0 g  
Yeast extract 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Glucose 5.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0-7.2  
Autoclave at 111°C for 30 min.

#### **65. OATMEAL AGAR FOR BACTERIA**

Oatmeal 2.0 g  
Peptone 0.5 g  
NaCl 1.0 g  
Galactose 0.5 g  
Agar 12.0 g  
Distilled water 1000.0 ml  
pH 7.5  
Autoclave at 111°C for 30 min.

#### **66. MEDIUM WITH SOIL EXTRACT**

Peptone 5.0 g  
Beef extract 3.0 g  
Agar 15.0 g  
Soil extract 250.0 ml  
Tap water 750.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

*Preparation of soil extract:* dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121°C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121°C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

#### **67. PEPTONE MEAT AGAR WITH TRACE ELEMENTS**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Yeast autolysate 2.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
Agar 20.0 g  
*Trace element solution:*  
H<sub>3</sub>BO<sub>3</sub> 5.0 g  
(NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> 5.0 g  
KI 0.5 g  
NaBr 0.5 g  
ZnSO<sub>4</sub> 0.2 g  
Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> 0.3 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

### **68. PEPTONE MEAT AGAR WITH 3% SEA SALT**

Peptone 10.0 g  
NaCl 5.0 g  
Sea salt 30.0 g  
Beef extract 3.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

### **69. DAVIS SUPPLEMENTED MINIMAL MEDIUM**

#### *Solution 1:*

Yeast extract 2.0 g  
Casein hydrolysate 2.0 g  
K<sub>2</sub>HPO<sub>4</sub> 7.0 g  
KH<sub>2</sub>PO<sub>4</sub> 3.0 g  
Na-citrate × 3 H<sub>2</sub>O 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
Agar 15.0 g  
Distilled water 980.0 ml

#### *Solution 2:*

Glucose 2.0 g  
Distilled water 20.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

### **70. MEDIUM VY/2 FOR STIGMATELLA AURANTIACA**

Baker's yeast 5.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.36 g  
Vitamin B<sub>12</sub> 0.5 mg  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2 (adjust with KOH before adding agar)  
Autoclave at 111°C for 30 min. Vitamin B<sub>12</sub> sterilize separately with filtration.

### **71. NITROSOCOCCUS MEDIUM 1**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.32 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 380.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 20.0 mg  
Chelated iron (13% iron) 1.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 100.0 мкг лучше μg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 200.0 μg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 μg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 μg  
K<sub>2</sub>HPO<sub>4</sub> 8.7 mg  
Phenol red (0.04%) 3.25 ml  
Sea water 1000.0 ml  
pH 7.5-7.8 (adjust with 1 N HCl)  
Autoclave at 121°C for 15 min.

### **72. BEAN AGAR**

Beans (peas or pulse) 100.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g

Sucrose 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
Boil beans in 1000.0 ml of water, filter through gauze, add water to the initial volume; add phosphate, sucrose and agar  
pH 7.2-7.4  
Autoclave at 111°C for 30 min.

### **73. GYT-AGAR**

Glucose 10.0 g  
Yeast extract 1.0 g  
Tryptose 2.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 mg  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 111°C for 30 min.

### **74. HOTTINGER BROTH**

Hottinger broth (HiMedia M1425) 23.0 g  
Autoclave at 121°C for 15 min.

### **75. MODIFICATION OF TWEEN-80 MEDIUM FOR MILK-ACID BACTERIA**

Yeast extract 5.0 g  
Glucose 2.5 g  
Beef extract 1.2 g  
Tween-80 1.0 ml  
K<sub>2</sub>HPO<sub>4</sub> 2.0 g  
Na-acetate 5.0 g  
NH<sub>4</sub>-citrate 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
MnSO<sub>4</sub> × 4 H<sub>2</sub>O 0.05 g  
Agar 5.0 g  
Distilled water 1000.0 ml  
pH 6.0-6.5  
Autoclave at 111°C for 30 min.

### **76. GLUCOSE MINERAL MEDIUM**

KNO<sub>3</sub> 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.04 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.04 g  
Na<sub>2</sub>HPO<sub>4</sub> 0.3 g  
KH<sub>2</sub>PO<sub>4</sub> 0.14 g  
Glucose 5.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Trace element solution:*  
Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Autoclave base medium and trace element solution at 121°C for 15 min.

#### **77. PEPTONE MEAT AGAR WITH VITAMINS**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Yeast extract 1.0 g  
Glucose 1.0 g  
Vitamin B<sub>12</sub> 2.0 mg  
Thiamin 2.0 mg  
Agar 20.0 g  
Distilled water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min.

#### **78. PEPTONE MEAT AGAR WITH 2% SOLUBLE STARCH**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 5.0 g  
Soluble starch 20.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min.

#### **79. PEPTONE MEAT AGAR WITH 1% SOLUBLE STARCH**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 5.0 g  
Soluble starch 10.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml

pH 7.2

Autoclave at 121°C for 15 min.

#### **80. PEPTONE MEAT AGAR WITH 6% NaCl**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 60.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min.



### **81. PEPTONE MEAT AGAR WITH 1.8% SEA SALT**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Sea salt 18.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

### **82. POTATO AGAR WITH 2% GLUCOSE**

Potato 200.0 g  
Glucose 20.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0  
Boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.  
Autoclave at 111°C for 30 min.

### **83. LOPATINA MEDIUM**

Glucose 10.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
Tyrosine 1.0 g  
NaCl 0.2 g  
CaSO<sub>4</sub> 0.1 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 111°C for 30 min

### **84. MEDIUM WITH CASEIN HYDROLYSATE**

Casein hydrolysate 10.0 g  
Glucose 5.0 g  
*p*-Aminobenzoic acid 5.0 mg  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 111°C for 30 min.

### **85. MEDIUM WITH HOTTINGER BROTH**

Peptone 10.0 g  
Yeast autolysate 10.0 g  
Hottinger broth (HiMedia M1425) 0.23 g  
Phosphate solution (see below) 25.0 ml  
Salt solution (see below) 25.0 ml  
Glucose 5.0 g  
Distilled water 950.0 ml  
*Phosphate solution:*  
KH<sub>2</sub>PO<sub>4</sub> 100.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 100.0 mg  
Distilled water 25.0 ml

*Salt solution:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 40.0 mg

NaCl 2.0 mg

FeSO<sub>4</sub> 2.0 mg

Distilled water 25.0 ml

pH 7.0

Autoclave phosphate and salt solutions at 121°C for 15 min, base medium at 111°C for 30 min.

**86. NITROSOLOBUS MEDIUM 1**

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

Chelated iron 1.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 mg

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.02 mg

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.65 g

K<sub>2</sub>HPO<sub>4</sub> 0.087 g

Phenol red 5.0 mg

Distilled water 1000.0 ml

pH 7.5 (adjust with 0.1 M NaHCO<sub>3</sub>)

Autoclave at 121°C for 15 min.

**87. NITROSOCOCCUS MEDIUM 2**

NH<sub>4</sub>Cl 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.05 g

CaCO<sub>3</sub> 5.0 g

Chelated iron 1.0 mg

Phenol red (0.04%) 3.25 ml

Sea water 1000.0 ml

pH 7.5-7.8 (adjust with 1 N HCl)

Autoclave at 121°C for 15 min.

**88. SPIRILLUM MEDIUM**

Peptone 10.0 g

Succinic acid 1.0 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g

FeCl<sub>3</sub> × 6 H<sub>2</sub>O 2.0 mg

MnSO<sub>4</sub> × H<sub>2</sub>O 2.0 mg

Distilled water 1000.0 ml

pH 6.8

Autoclave at 121°C for 15 min.

**89. MILK MEDIUM FOR HALOPHILS**

*Solution 1:*

Milk 500.0 ml

*Solution 2:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 g

KNO<sub>3</sub> 2.0 g

NaCl 200.0 g

Distilled water 100.0 ml

*Solution 3:*

Peptone 5.0 g  
Glycerol 10.0 g  
Agar 25.0 g  
Distilled water 400.0 ml  
pH 8.4  
Autoclave at 121°C for 15 min.  
Sequence of mixing: add warm skim milk to a hot mixture of solutions 1 and 2.

### **90. *DESULFOVIBRIO GIGAS* MEDIUM**

#### *Solution 1:*

$\text{KH}_2\text{PO}_4$  1.0 g  
 $\text{NH}_4\text{Cl}$  0.5 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.4 g  
 $\text{Na}_2\text{SO}_4$  2.0 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g  
Trace element solution *SL-6*: (see below) 1.0 ml  
2 M  $\text{H}_2\text{SO}_4$  1.0 ml  
Na L-lactate 2.0 g  
Distilled water 950.0 ml

#### *Solution 2:*

$\text{NaHCO}_3$  2.0 g  
Distilled water 40.0 ml

#### *Solution 3:*

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  300.0 mg  
Distilled water 6.0 ml

#### *Vitamin solution:*

Biotin 2.5 mg  
Nicotinic acid 25.0 mg  
Thiamine-HCl 12.5 mg  
*p*-Aminobenzoic acid 12.5 mg  
Calcium pantothenate 6.5 mg  
Pyridoxine-HCl 62.5 mg  
Distilled water 1000.0 ml

#### *Trace element solution SL-6:*

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  0.03 g  
 $\text{H}_3\text{BO}_3$  0.3 g  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  0.2 g  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  0.01 g  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.02 g  
 $\text{Na}_2\text{MoO}_4$  0.03 g  
Distilled water 1000.0 ml  
pH 7.2

Autoclave solutions 1 and 3 separately under  $\text{N}_2$  at 121°C for 15 min, add 5.0 ml of the filter sterilized vitamin solution to 1000.0 ml of sterile solution 1. Solution 2 (autoclave at 121°C for 15 min) is not to be kept for long.

### **91. *THERMODESULFOBACTERIUM* MEDIUM**

#### *Solution 1:*

$\text{Na}_2\text{SO}_4$  3.0 g  
 $\text{NH}_4\text{Cl}$  1.0 g  
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.2 g  
 $\text{KH}_2\text{PO}_4$  0.3 g  
 $\text{Na}_2\text{HPO}_4 \times 12 \text{H}_2\text{O}$  2.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.5 mg

Resazurin 1.0 mg

Distilled water 930.0 ml

*Solution 2:*

Trace element solution (see below) 10.0 ml

*Solution 3:*

Yeast extract 1.0 g

Distilled water 25.0 ml

*Solution 4:*

Na-lactate 4.0 g

Distilled water 25.0 ml

*Solution 5:*

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

Distilled water 6.0 ml

*Solution 6:*

Vitamin solution (see below) 5.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 g

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.17 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

ZnCl<sub>2</sub> 0.1 g

CuCl<sub>2</sub> 0.02 g

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

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.026 g

NaCl 1.0 g

Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 6.8-7.0

Solutions 1, 3, 4 and 5 autoclave under N<sub>2</sub> at 121°C for 15 min.

Solution 6 is filter sterilized. Before use, neutralize solution 5 by dropwise of 1 N HCl.

## **92. DESULFOVIBRIO MEDIUM WITH LACTATE**

*Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

NH<sub>4</sub>Cl 1.0 g

CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

Na<sub>2</sub>SO<sub>4</sub> 1.0 g

Na-lactate 5.0 g

Yeast extract 1.0 g

Resazurin 0.01 g  
Cysteine 0.5 g  
Distilled water 950.0 ml

*Solution 2:*

NaHCO<sub>3</sub> 4.0 g  
Distilled water 40.0 ml

*Solution 3:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 300.0 mg  
Distilled water 6.0 ml

*Solution 4:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

pH 6.8

Autoclave all solutions separately at 121°C for 15 min. Solution 1 bring to boil while simultaneously bubbling a mixture of oxygen-free gas composed of 97% N<sub>2</sub> and 3% H<sub>2</sub> through the mixture and sterilize in atmosphere of this gas mixture. Solution 3 sterilize in atmosphere of N<sub>2</sub>.

### **93. AZOSPIRILLUM BRASILIENSE MEDIUM 1**

Ca-malate 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml

pH 6.5

Autoclave at 121°C for 15 min.

### **94. MEDIUM FOR CARBON MONOOXIDE OXIDIZERS**

Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 4.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.75 g  
NH<sub>4</sub>Cl 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g  
Fe(NH<sub>4</sub>)-citrate 0.018 g  
Agar (if necessary) 1.2 g  
Trace element solution *SL-6* (see below) 1.0 ml  
Vitamin solution (see below) 10.0 ml  
Distilled water 1000.0 ml

*Trace element solution SL-6:*

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.0

Autoclave at 121°C for 15 min, add vitamin solution sterilized by filtration. For chemoautotrophic growth incubate under gas atmosphere of a) 20-80% CO<sub>2</sub> + 10% O<sub>2</sub> + 0-70% N<sub>2</sub> or b) 70% H<sub>2</sub> + 20% O<sub>2</sub> + 10% CO<sub>2</sub> adding 2.5 g NaHCO<sub>3</sub> per liter of medium. For chemoorganotrophic growth add 3.0 g sodium acetate and incubate under air.

### **95. DESULFOTOMACULUM ACETOXIDANS MEDIUM**

#### *Solution 1:*

NaCl 1.17 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KCl 0.3 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
NH<sub>4</sub>Cl 0.27 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 2.84 g  
Na-acetate 1.4 g  
Na-butyrate 1.4 g  
Yeast extract 1.0 g  
Vitamin solution (see below) 1.0 ml  
Trace element solution 1.0 ml (see below)  
Distilled water 940.0 ml

#### *Solution 2:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.36 g  
Distilled water 10.0 ml

#### *Sodium bicarbonate for alkalization:*

NaHCO<sub>3</sub> 4.5 g  
Distilled water 50.0 g

#### *Vitamin solution:*

*p*-Aminobenzoic acid 4.0 mg  
D(+)-Biotin 1.0 mg  
Thiamine-HCl 10.0 mg  
Distilled water 100.0 ml

#### *Trace element solution:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 ml  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 120.0 ml  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
HCl (0.05 M) 1000.0 ml  
pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9 - 7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Vitamin solution is filter sterilized.

### **96. DESULFONEMA LIMICOLA MEDIUM**

#### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
NaCl 13.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 2.2 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g

KCl 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

Distilled water 850.0 ml

*Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g

Distilled water 100.0 ml

*Solution 4:*

Na-acetate × 3 H<sub>2</sub>O 2.5 g

Distilled water 10.0 ml

*Solution 5:*

Disodium succinate 0.1 g

Distilled water 1.0 ml

*Solution 6:*

Vitamin solution (see below) 5.0 ml

*Solution 7:*

AlCl<sub>3</sub> × 6 H<sub>2</sub>O 245.0 mg

Distilled water 5.0 ml

*Solution 8:*

Na<sub>2</sub>CO<sub>3</sub> 170.0 mg

Distilled water 1.6 ml

*Solution 9:*

Rumen fluid, clarified 20.0 ml

*Solution 10:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 mg

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.6

*The trace element solution preparation:*  $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated. Solution 1 is boiled for a few minutes, cooled to room temperature, gassed with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  gas mixture to reach a pH of around 6, then autoclaved anaerobically under the same gas. Solutions 2, 3, 5, 9, and 10 are autoclaved separately under nitrogen at 121°C for 15 min, solution 3 is filter-sterilized and flushed with 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  to remove dissolved oxygen. Solution 6 is filter-sterilized and outgassed with  $\text{N}_2$ . Solutions 7 and 8 are combined before autoclaving at 121°C for 15 min. Solutions 2 to 10 are added to the sterile cooled solution A in the sequence as indicated. The completed medium is distributed anaerobically under 80%  $\text{N}_2$  + 20%  $\text{CO}_2$  into appropriate vessels.

### **97. DESULFONEMA MAGNUM MEDIUM**

#### *Solution 1:*

$\text{Na}_2\text{SO}_4$  3.0 g  
 $\text{NaCl}$  21.0 g  
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  5.5 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  1.35 g  
 $\text{KCl}$  0.5 g  
 $\text{KH}_2\text{PO}_4$  0.2 g  
 $\text{NH}_4\text{Cl}$  0.3 g  
Distilled water 850.0 ml

#### *Solution 2:*

Trace element solution (see below) 1.0 ml

#### *Solution 3:*

$\text{NaHCO}_3$  2.5 g  
Distilled water 100.0 ml

#### *Solution 4:*

Na-benzoate 0.6 g  
Distilled water 10.0 ml

#### *Solution 5:*

Disodium succinate 0.1 g  
Distilled water 1.0 ml

#### *Solution 6:*

Vitamin solution (see below) 5.0 ml

#### *Solution 7:*

$\text{AlCl}_3 \times 6 \text{H}_2\text{O}$  245.0 mg  
Distilled water 5.0 ml

#### *Solution 8:*

$\text{Na}_2\text{CO}_3$  170.0 mg  
Distilled water 1.6 ml

#### *Solution 9:*

Rumen fluid, clarified 20.0 ml

#### *Solution 10:*

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.4 mg  
Distilled water 10.0 ml

#### *Trace element solution:*

HCl (25%; 7.7 M) 10.0 ml  
 $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.5 g  
 $\text{ZnCl}_2$  70.0 mg  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg  
 $\text{H}_3\text{BO}_3$  6.0 mg  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  36.0 mg



Na<sub>2</sub>SeO<sub>4</sub> 3.0 mg  
Distilled water 990.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Vitamin B<sub>12</sub> 50.0 mg  
Distilled water 1000.0 ml  
pH 7.6

*The trace element solution preparation:* FeCl<sub>2</sub> × 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated. Solution 1 is boiled for a few minutes, cooled to room temperature, gassed with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH of around 6, then autoclaved anaerobically under the same gas. Solutions 2, 3, 5, 9, and 10 are autoclaved separately under nitrogen at 121°C for 15 min, solution 3 is filter-sterilized and flushed with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen. Solution 6 is filter-sterilized and outgassed with N<sub>2</sub>. Solutions 7 and 8 are combined before autoclaving at 121°C for 15 min. Solutions 2 to 10 are added to the sterile cooled solution A in the sequence as indicated. The completed medium is distributed anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels.

#### **98. WORT AGAR 7 B WITH 2% CaCO<sub>3</sub>**

Malt extract Balling 7 degrees 1000.0 ml  
CaCO<sub>3</sub> 20 g  
Agar 20.0 g  
Autoclave at 111°C for 30 min.

#### **99. MEDIUM YE**

Yeast extract 30.0 g  
Ethanol 20.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 5.0-6.0  
Autoclave at 121°C for 15 min without ethanol.  
Apply filter sterilize ethanol (0.1 ml/test tube) onto the surface of agar slants.

#### **100. SAP-2 AGAR**

Tryptone 1.0 g  
Yeast extract 1.0 g  
Agar 20.0 g  
Sea water 1000.0 g  
pH 7.4  
Autoclave at 121°C for 15 min.

#### **101. SOIL AGAR**

Yeast extract 2.0 g  
Tryptone 1.0 g  
Na-acetate 1.0 g  
Soil extract 50.0 ml  
Agar 20.0 g

Distilled water add to 1000.0 ml

pH 7.4

Autoclave at 121°C for 15 min.

*Preparation of soil extract:* dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121°C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121°C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

## **102. DESULFOBACTER POSTGATEI MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

NaCl 7.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 1.3 g

KCl 0.5 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g

Distilled water 870.0 ml

*Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g

Distilled water 100.0 ml

*Solution 4:*

Na-acetate × 3 H<sub>2</sub>O 2.5 g

Distilled water 10.0 ml

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

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

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Vitamin solution is filter sterilized.

### **103. *DESULFOBULBUS* MEDIUM**

#### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

#### *Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

#### *Solution 3:*

NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

#### *Solution 4:*

Na-propionate 1.5 g  
Distilled water 10.0 ml

#### *Solution 5:*

Vitamin solution (see below) 10.0 ml

#### *Solution 6:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Vitamin solution is filter sterilized.

#### **104. MACROMONAS MEDIUM 1**

Na-acetate 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
Casein acidic hydrolysate 0.1 g  
Yeast extract 0.1 g  
FeS or CaS 0.2 g  
Agar 1.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4

Prepare suspension of FeS separately from the equimolar solutions of Na<sub>2</sub>S × 9 H<sub>2</sub>O and FeSO<sub>4</sub>, wash with freshly boiled distilled water under the flow of inert gas, sterilize separately from the main medium under inert gas at 111°C for 30 min. Main medium autoclave at 121°C for 15 min.

#### **105. MACROMONAS MEDIUM 2**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
Casein acidic hydrolysate 1.0 g  
Na-acetate × 3 H<sub>2</sub>O 1.0 g  
or succinate, or benzoate 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 0.5 g  
Catalase 2.0 mg  
Vitamin solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Autoclave at 121°C for 15 min.

Sterilize catalase and vitamins separately from the main medium by filtration. Thiosulfate should also better be autoclaved separately at 121°C for 15 min.

#### **106. BEGGIATOIA MEDIUM 1**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 500.0 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 50.0 mg  
Na-lactate 500.0 mg  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 500.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 110.0 mg  
KH<sub>2</sub>PO<sub>4</sub> 85.0 mg  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O 2.0 mg

EDTA 3.0 mg  
Vitamin solution (see below) 1.0 ml  
Buffer HEPES 0.01 M  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.5 (adjust with NaOH)

Sterilize vitamins (by filtration); main medium, thiosulfate and lactate each separately (autoclave at 121°C for 15 min) and add into the main medium prior to inoculation.

### **107. DESULFOSARCINA MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 13.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

*Solution 2:*

Trace element solution (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

*Solution 4:*

Na-benzoate 0.6 g  
Na-lactate 1.0 g  
Distilled water 10.0 ml

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

*Trace element solution:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3.0 mg

Distilled water 990.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Vitamin solution is filter sterilized.

### **108. BEGGIATOIA MEDIUM 2**

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 50.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 30.0 mg  
Na-lactate 500.0 mg  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 500.0 mg  
NaH<sub>2</sub>PO<sub>4</sub> 125.0 mg  
KCl 125.0 mg  
NaHCO<sub>3</sub> 125.0 mg  
Na<sub>2</sub>SO<sub>4</sub> 500.0 mg  
NaNO<sub>3</sub> 620.0 mg  
Vitamin solution (see below) 1.0 ml  
Trace element solution according to *Hogland*: (see below) 1.0 ml  
Distilled water 1000.0 ml

*Vitamin solution:*  
Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> 10.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml

pH 7.5 (adjust with 1% HCl)

To prepare the trace element solution, preliminarily acidify water to pH 3.0-4.0 with HCl. Sterilize thiosulfate, lactate, bicarbonate, trace elements and vitamins separately and add to the main medium prior to inoculation. Lactate and thiosulfate can be more conveniently prepared as 10% solutions; bicarbonate, as 5% solution. Sterilize the vitamin solution by filtration, others solutions and base medium by autoclaving at 121°C for 15 min.

### **109. *DESULFOVIBRIO* MEDIUM**

#### *Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
Na<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
Na DL-lactate 2.0 g  
Yeast extract 1.0 g  
Resazurin 1.0 g  
Distilled water 980.0 ml

#### *Solution 2:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

#### *Solution 3:*

Na-thioglycollate 0.1 g  
Ascorbic acid 0.1 g  
Distilled water 10.0 ml

#### *Solution 4:*

Trace element solution (see below) 1.0 ml

#### *Trace element solution:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 ml  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 120.0 ml  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
HCl, 0.05 M 1000.0 ml  
pH 7.4-7.8

Autoclave at 121°C for 15 min under an atmosphere of N<sub>2</sub>. Solution 1 is boiled before sterilization, being blown with N<sub>2</sub>.

### **110. *DESULFOVIBRIO* MEDIUM WITH 2% NaCl**

#### *Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
NaCl 20 g  
Na<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
Na DL-lactate 2.0 g  
Yeast extract 1.0 g  
Resazurin 1.0 mg  
Distilled water 980.0 ml

#### *Solution 2:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

*Solution 3:*

Na-thioglycollate 0.1 g  
Ascorbic acid 0.1 g  
Distilled water 10.0 ml

*Solution 4:*

Trace element solution (see below) 1.0 ml

*Trace element solution:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 120.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
HCl, 0.05 M 1000.0 ml  
pH 7.4-7.8

Autoclave at 121°C for 15 min under an atmosphere of N<sub>2</sub>. Solution 1 is boiled before sterilization, being blown with N<sub>2</sub>.

**111. DESULFOVIBRIO MEDIUM WITH 3% NaCl**

*Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
NaCl 30 g  
Na<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
Na DL-Lactate 2.0 g  
Yeast extract 1.0 g  
Resazurin 1.0 mg  
Distilled water 980.0 ml

*Solution 2:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

*Solution 3:*

Na-thioglycollate 0.1 g  
Ascorbic acid 0.1 g  
Distilled water 10.0 ml

*Solution 4:*

Trace element solution (see below) 1.0 ml

*Trace element solution:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 120.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
HCl, 0.05 M 1000.0 ml  
pH 7.4-7.8

Autoclave at 121°C for 15 min under an atmosphere of N<sub>2</sub>. Solution 1 is boiled before sterilization, being blown with N<sub>2</sub>.

**112. GLUCOSE YEAST EXTRACT AGAR**

Glucose 20.0 g  
Yeast extract 10.0 g



CaCO<sub>3</sub> 20.0 g  
Agar 17.0 g  
Distilled water 1000.0 ml  
Autoclave at 111°C for 30 min.

### **113. *DESULFOVIBRIO BAARSII* MEDIUM**

#### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

#### *Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

#### *Solution 3:*

NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

#### *Solution 4:*

Na-butyrate 0.7 g  
Na-caproate 0.3 g  
Na-octanoate 0.15 g  
Distilled water 10.0 ml

#### *Solution 5:*

Vitamin solution (see below) 10.0 ml

#### *Solution 6:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 6.8-7.0

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Vitamin solution is filter sterilized.

#### **114. AZOSPIRILLUM BRASILIENSE MEDIUM 2**

Ca-malate 10.0 g or  
Glucose 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.02 mg  
Yeast extract 0.1 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 6.9  
Autoclave at 111°C for 30 min.

#### **115. METHANOBACTERIUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g  
NaCl 0.4 g  
NH<sub>4</sub>Cl 0.4 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Vitamin solution (see below) 5.0 ml  
Yeast extract 1.0 g  
Na-acetate 1.0 g  
Na-formate 2.0 g  
NaHCO<sub>3</sub> 4.0 g  
Resazurin 1.0 mg  
L-Cysteine-HCl 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Fatty acid mixture (see below) 20.0 ml  
Sludge water: (see below) 50.0 ml  
Distilled water 940.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Fatty acid mixture:*

Valeric acid 0.5 g  
Isovaleric acid 0.5 g  
 $\alpha$ -Methylbutyric acid 0.5 g  
Isobutyric acid 0.5 g  
Distilled water 20.0 ml

pH 7.5 (adjust with concentrated NaOH)

*Sludge water:* to sludge from an anaerobic digester add 0.4% yeast extract and after gassing with N<sub>2</sub> gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13,000 g and autoclave the resulting clear supernatant under N<sub>2</sub> gas at 121°C for 15 min (pH 6.7-7.0). Base medium and trace element solution autoclave at 121°C for 15 min. Vitamin solution is filter sterilized. Prepare the medium anaerobically under a gas atmosphere of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

### **116. THERMUS RUBER MEDIUM**

Pepton 5.0 g  
Yeast extract 1.0 g  
Starch (soluble) 1.0 g  
Agar 12.0 g  
Distilled water 1000.0 ml  
pH 8.0  
Autoclave at 121°C for 15 min.

### **117. METHANOSARCINA MEDIUM**

*Solution 1:*

NaCl 0.9 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
Yeast extract 2.0 g  
Resazurin 10.0 mg  
Methanol 10% 10.0 ml  
Trace element solution (see below) 10.0 ml  
Vitamin solution (see below) 5.0 ml  
Distilled water 965.0 ml

*Solution 2 (reducing agents):*

L-Cysteine-HCl 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

*Buffer solutions:*

a) K<sub>2</sub>HPO<sub>4</sub> 29.0 g  
Distilled water 100.0 ml  
b) KH<sub>2</sub>PO<sub>4</sub> 15.0 g  
Distilled water 100.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g

CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnCl<sub>2</sub> 0.1 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NaCl 1.0 g  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 g  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization. Solutions of reducing agents (10.0 ml) and of buffer (per 1 ml) add to base medium after separate sterilization. Base medium, trace elements, buffer solutions and reducing agents autoclave at 121°C for 15 min. Vitamin solution is filter sterilized.

### **118. PLATE COUNT AGAR**

Tryptone 5.0 g  
Yeast extract 2.5 g  
Glucose 1.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.0±0.2  
Autoclave at 121°C for 15 min.

### **119. NITROSOLOBUS MEDIUM 2**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.04 g  
EDTA FeNa 3.8% 0.1 ml  
Phenol red 0.05% 2.0 ml  
Distilled water 1000.0 ml  
pH 8.0 (adjust with 6% Na<sub>2</sub>CO<sub>3</sub>)  
Autoclave at 121°C for 15 min.

### **120. MANNITOL AGAR WITH PEPTONE**

Yeast extract 5.0 g  
Mannitol 25.0 g  
Peptone 3.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 5.0-6.0

Autoclave at 121°C for 15 min.

**121. *THIOBACILLUS THIOOXIDANS* MEDIUM (WAKSMAN MEDIUM)**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 300.0 mg

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg

KH<sub>2</sub>PO<sub>4</sub> 3.5 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 250.0 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 18.0 mg

Finely dispersed sulfur 5.0 g

Distilled water 1000.0 ml

pH 4.5

Autoclave at 121°C for 15 min.

**122. *THIOBACILLUS FERROOXIDANS* MEDIUM (LEATHEN MEDIUM)**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 150.0 mg

KCl 50.0 mg

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg

KH<sub>2</sub>PO<sub>4</sub> 100.0 mg

Ca(NO<sub>3</sub>)<sub>2</sub> × 4 H<sub>2</sub>O 10.0 mg

Distilled water 1000.0 ml

Autoclave at 121°C for 15 min.

After sterilization of the medium, add 10.0 ml of 10% FeSO<sub>4</sub> × 7 H<sub>2</sub>O preliminarily acidified to pH 3.5 and autoclaved separately in sealed ampoules under nitrogen. pH 4.0 (adjust after sterilization and addition of iron solution).

**123. MEDIUM FOR MARINE *NITROBACTER***

Solution 1 (see below) 0.5 ml

Solution 2 (see below) 0.5 ml

Solution 3 (see below) 1.0 ml

Solution 4 (see below) 0.5 ml

Solution 5 (see below) 0.5 ml

Solution 6 (see below) 0.1 ml

Distilled water 300.0 ml

Sea water 700.0 ml

*Solution 1:*

CaCl<sub>2</sub> 2.0 g

Distilled water 100.0 ml

*Solution 2:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g

Distilled water 100.0 ml

*Solution 3:*

Chelated iron 0.1 g

Distilled water 100.0 ml

*Solution 4:*

Na<sub>2</sub>MoO<sub>4</sub> 0.1 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

*Solution 5:*

NaNO<sub>3</sub> 41.4 g

Distilled water 100.0 ml

*Solution 6:*

K<sub>2</sub>HPO<sub>4</sub> 1.74 g  
Distilled water 100.0 ml  
pH 8.6 (adjust with NaOH or KOH)  
Autoclave all solutions separately at 121°C for 15 min. Leave the medium to stand for 2–3 days so that pH can adjust itself to pH 7.4-7.6.

#### **124. AZOSPIRILLUM MEDIUM**

##### *Solution 1:*

Yeast extract 0.05 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
K<sub>2</sub>HPO<sub>4</sub> 0.25 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 1.0 mg  
MnSO<sub>4</sub> × H<sub>2</sub>O 2.0 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
NaCl 0.1 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
Biotin 0.1 mg  
Bromothymol blue 25.0 mg  
Distilled water 950.0 ml

##### *Solution 2:*

Glucose 20% 25.0 ml

##### *Solution 3:*

Na-malate 20% 25.0 ml

Dissolve bromothymol blue in diluted KOH before adding into the medium.  
pH 7.1

Autoclave solutions separately at 121°C for 15 min.

#### **125. NITROSOLOBUS MEDIUM 3**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 20.0 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 200.0 mg  
Chelated iron 1.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 200.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 15.9 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml  
pH 7.5-7.8  
Autoclave at 121°C for 15 min.

#### **126. DESULFOCOCCUS NIACINI MEDIUM**

##### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 13.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 2.2 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

##### *Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g

Distilled water 100.0 ml

*Solution 4:*

Na-nicotinate 5.0 mM

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

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

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 40.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Trace element solution is autoclaved at 121°C for 15 min. Vitamin solution is filter sterilized.

Final pH of the complete medium 7.4

## **127. SELENITE CONTROL MEDIUM**

*Solution 1:*

Peptone 5.0 g

Na<sub>2</sub>HPO<sub>4</sub> 7.0 g

NaH<sub>2</sub>PO<sub>4</sub> 3.0 g

Lactose 4.0 g

Distilled water 960.0 ml

pH 6.9-7.1

*Solution 2:*

10% Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 40.0 ml

Autoclave at 111°C for 30 min.

### **128. HIRSCH MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 1.36 g  
Na<sub>2</sub>HPO<sub>4</sub> 2.15 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
MnSO<sub>4</sub> × 5 H<sub>2</sub>O 1.05 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 5.97 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 1.5 mg  
Methanol 5.0 ml  
Distilled water to 1000.0 ml  
pH 7.0

Autoclave at 121°C for 15 min. Sterilize methanol by filtration and add to the medium after autoclaving.

### **129. CONTROL MEDIUM C-1**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
K<sub>2</sub>HPO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
NaCl 5.0 g  
Raffinose 2.0 g  
Bromothymol blue (1.6% alkaline) 2.0 ml  
Crystal violet (0.01%) 20.0 ml  
50% Urea 4.0 ml  
Distilled water 975.0 ml  
Autoclave at 111°C for 30 min. Urea solution is filter sterilized.

### **130. POTATO-GLUCOSE AGAR**

Potato 200.0 g  
Glucose 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0  
Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.  
Autoclave at 111°C for 30 min.

### **131. MEDIUM Q MOD FOR *FRANKIA***

K<sub>2</sub>HPO<sub>4</sub> 300.0 mg  
NaH<sub>2</sub>PO<sub>4</sub> 200.0 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 200.0 mg  
KCl 200.0 mg  
Yeast extract 500.0 mg  
Peptone 5.0 g  
Glucose 10.0 g  
Fe-citrate 1.0 ml  
Trace element solution (see below) 1.0 ml  
CaCO<sub>3</sub> 100.0 mg  
Tween-80 2.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
*Trace element solution:*  
H<sub>3</sub>BO<sub>3</sub> 1.5 g  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.8 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.6 g



CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.1 g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> × 4 H<sub>2</sub>O 0.2 g  
CoSO<sub>4</sub> 0.01 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 111°C for 30 min.

### **132. THIOBACILLUS FERROOXIDANS MEDIUM 9K**

*Solution 1:*

KCl 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Ca(NO<sub>3</sub>)<sub>2</sub> × 4 H<sub>2</sub>O 0.01 g  
Distilled water 700.0 ml

*Solution 2:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> 63.0 g  
H<sub>2</sub>SO<sub>4</sub> (10 N) 1.0 ml  
Distilled water 300.0 ml  
pH 3.5

Autoclave solution 1 at 121°C for 15 min, solution 2 at 111°C 30 min. Mix the solutions before inoculation.

### **133. COLBY AND ZATMAN MEDIUM WITH METHANOL**

K<sub>2</sub>HPO<sub>4</sub> 1.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.62 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.05 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
NaCl 0.1 g  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O 1.0 mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 5.0 µg  
MnSO<sub>4</sub> × 5 H<sub>2</sub>O 10.0 µg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 10.0 µg  
H<sub>3</sub>BO<sub>3</sub> 10.0 µg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 70.0 µg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 5.0 µg  
Purified agar 15.0 g  
Methanol 1.0 ml  
Distilled water 1000.0 ml  
pH 6.8

Autoclave at 121°C for 15 min. Methanol is filter sterilized.

### **134. METHYLOTROPH MEDIUM 1**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Methanol 5.0 ml  
Distilled water 1000.0 ml  
pH 7.0

Autoclave at 121°C for 15 min. Methanol is filter sterilized. Methanol may be replaced 30 ml 10 % solution of methylamine, autoclave at 111°C for 30 min.

### 135. METHYLOTROPH MEDIUM 2

KH<sub>2</sub>PO<sub>4</sub> 0.8 g  
Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 3.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.8 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 mg  
Methanol 5.0 ml  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Trace element solution:*

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.25 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 1.25 g  
MnSO<sub>4</sub> × 4 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1.25 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 50.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 250.0 mg  
Distilled water 250.0 ml  
pH 7.0-7.2

Autoclave at 121°C for 15 min. Methanol is filter sterilized. Methanol may be replacement 30 ml 10 % solution of methylamine, autoclave at 111°C for 30 min.

### 136. MEDIUM FOR PERCHLORATE-REDUCING BACTERIA

*Solution 1:*

NH<sub>4</sub>Cl 0.1 g  
NaCl 0.02 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
K<sub>2</sub>HPO<sub>4</sub> 0.4 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Tap water 1000.0 ml

*Solution 2:*

HCl, to dissolve the precipitate in solution 1

*Solution 3:*

pH 6.9-7.2 (adjust with 5% NaOH)

*Solution 4:*

5% NH<sub>4</sub>ClO<sub>4</sub> 5.0 ml

*Solution 5:*

5% Na-acetate 5.0 ml

*Solution 6:*

Trace element solution according to *Hogland* (see below) 0.5 ml

*Solution 7:*

Vitamin B<sub>12</sub> (dispensary solution) 0.2 ml

*Solution 8:*

96° ethanol 0.5 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml

Sterilize ethanol by filtration, others solutions separately autoclave at 121°C for 15 min. Add solutions and additions to the main medium in the order of their enumeration.

### **137. MEDIUM FOR CHROMATE-REDUCING BACTERIA**

*Solution 1:*

NH<sub>4</sub>Cl 0.3 g  
NaCl 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.3 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CaCO<sub>3</sub> 0.05 g  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O 0.05 g  
K<sub>2</sub>CrO<sub>4</sub> 0.1 g  
Paper-filtered pond water 900.0 ml

*Solution 2:*

Peptone meat broth 100.0 ml

*Solution 3:*

5% Na-acetate 5.0 ml

*Solution 4:*

Trace element solution according to *Hogland* (see below) 0.5 ml

*Solution 5:*

Vitamin B<sub>12</sub> (dispensary solution) 0.2 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml  
pH 7.0-7.2

Autoclave solutions separately at 121°C for 15 min. Add solutions and additions to the main medium in the order of their enumeration.

### **138. LARSEN PHOTOTROPH MEDIUM**

NH<sub>4</sub>Cl 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
MgCl<sub>2</sub> 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
Trace element solution SL-12B (see below) 1.0 ml  
NaHCO<sub>3</sub> 5.0 g  
Na-acetate × 3 H<sub>2</sub>O 2.0 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.4-0.6 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.1 g  
Fe-citrate – traces  
Distilled water 1000.0 ml

*Trace element solution SL-12B:*

EDTA Na 3.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.1 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
MnCl<sub>2</sub> × 2 H<sub>2</sub>O 50.0 mg

ZnCl<sub>2</sub> 42.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 18.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Distilled water 1000.0 ml  
pH of the trace element solution 6.0  
pH 8.4  
Autoclave solutions separately at 121°C for 15 min.

### **139. POSTGATE MEDIUM B FOR SULFATE REDUCERS**

#### *Solution 1:*

NaCl 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
CaSO<sub>4</sub> × 2 H<sub>2</sub>O 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
Na-lactate 3.5 g  
Yeast extract 1.0 g  
Tap water 980.0 ml  
Dissolve ingredients and sparge medium with O<sub>2</sub> free N<sub>2</sub> gas for 10-15 minutes

#### *Solution 2:*

Ascorbic acid 1.0 g  
Thioglycolic acid 1.0 g  
Distilled water 10.0 ml

#### *Solution 3:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml  
pH 7.4

Autoclave solution 1 at 121°C for 15 min, solutions 2 and 3 separately autoclave at 111°C for 30 min.

### **140. DILUTED BRAIN HEART INFUSION MEDIUM (DILUTED BHI MEDIUM)**

BHI broth (Pronadisa 1400) 3.7 g or  
Peptone mixture 1.0 g  
Beef heart infusion 1.0 g  
Calf brain infusion 0.75 g  
Glucose 0.2 g  
Na<sub>2</sub>HPO<sub>4</sub> 0.25 g  
NaCl 0.5 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.4 ± 0.2  
Autoclave at 121°C for 15 min.

### **141. CITRATE AGAR**

NaCl 5.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
Na-citrate 3.0 g  
Bromothymol blue 0.08 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2

Autoclave at 121°C for 15 min.

#### **142. PPYA**

Potato decoction (see below) 200.0 ml

Peptone 5.0 g

Yeast extract 1.0 g

Agar 25.0 g

Distilled water to 1000.0 ml

pH 8.0.

Autoclave at 121°C for 15 min

*Preparation of potato decoction:* boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume. Do not use new potatoes.

#### **143. PEPTONE MEAT AGAR WITH 1% GLYCEROL**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Glycerol 10.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0-7.2

Autoclave at 121°C for 15 min.

#### **144. PEPTONE MEAT AGAR WITH 0.5% GLUCOSE**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Glucose 5.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0-7.2

Autoclave at 111°C for 30 min

#### **145. PSEUDOMONAS SP. (ARTHROBACTER GLOBIFORMIS) MEDIUM**

Glucose 10.0 g

Maize extract 10.0 g

Tap water 1000.0 ml

pH 7.8 (adjust with NH<sub>4</sub>OH); paper filter.

Autoclave at 111°C for 30 min.

#### **146. DILUTED TRYPTICASE SOY MEDIUM**

Trypticase Soy Broth (BBL 11768, Oxoid CM129 or Merck 5459) 3.0 g or

Pancreatic digest of casein 1.8 g

Papaic digest of soyabean 0.6 g

NaCl 0.6 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.3

Autoclave at 121°C for 15 min.

#### **147. HYPHOMICROBIUM MEDIUM**

NaNO<sub>3</sub> 1.0 g

NaCl 0.5 g

K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
Methanol 5.0-10.0 ml  
Distilled water 1000.0 ml  
pH 6.8-7.0

Autoclave at 121°C for 15 min. Methanol is filter sterilized.  
Grow in an exicator in the presence of methanol vapors.

#### **148. GLUCOSE YEAST CHALK MEDIUM**

Beef extract 3.0 g  
Yeast extract 10.0 g  
Glucose 50.0 g  
CaCO<sub>3</sub> 30.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
Autoclave at 121°C for 15 min.

#### **149. CLAVIBACTER XYLI MEDIUM**

*Solution 1:*

Flour agar 5.0 g  
Papaya hydrolysate of soybean meal 8.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
Distilled water 965.0 ml

*Solution 2:*

Bovine hemine chloride (0.1% in 0.05 N NaOH) 15.0 ml

*Solution 3:*

Bovine serum albumin, fraction 5 (20%) 10.0 ml

*Solution 4:*

Glucose (50%) 1.0 ml

*Solution 5:*

Cysteine (10%) 10.0 ml

pH 6.6

Autoclave solution 1 at 121°C for 15 min, cool to 50 C and add the filter-sterilized solutions 2-5.

#### **150. GETCHINSON MEDIUM WITH FILTER PAPER**

K<sub>2</sub>HPO<sub>4</sub> 1.3 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.3 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O 0.01 g  
NaNO<sub>3</sub> 2.5 g  
Distilled water 1000.0 ml  
pH 7.2-7.3.

Autoclave at 121°C for 15 min. Cut filter paper into strips, sterilize by dry heat and immerse into the medium so that they are not completely in the liquid medium.

#### **151. MUNZ MEDIUM FOR METHANE-OXIDIZING BACTERIA**

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
Tap water 1000.0 ml  
pH 6.8

Autoclave at 121°C for 15 min.

Cultivate in the mixed atmosphere of air and methane (2:1).

### **152. TRYPTONE THIOGLYCOLATE MEDIUM**

*Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 5.45 g

KH<sub>2</sub>PO<sub>4</sub> 1.2 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 25.0 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 15.0 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 2.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 2.5 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 2.5 mg

Peptone 2.0 g

Tryptone 2.0 g

Yeast extract 6.0 g

Na-thioglycolate 0.5 g

Distilled water 950.0 ml

*Solution 2:*

Glucose 20.0 g

Distilled water 50.0 ml

pH 7.5

Autoclave solution 1 at 121°C for 15 min, solution 2 at 111°C for 30 min.

### **153. MEDIUM P-2 FOR THERMOPHILIC ANAEROBIC BACTERIA**

K<sub>2</sub>HPO<sub>4</sub> 3.0 g

KH<sub>2</sub>PO<sub>4</sub> 2.0 g

NH<sub>4</sub>Cl 2.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g

Tryptone 10.0 g

Glucose 5.0 g

Yeast extract 5.0 g

Resazurin 0.01 g

Distilled water 1000.0 ml

pH 7.0-7.2

Prepare medium anaerobically under N<sub>2</sub> and autoclave at 121°C for 15 min. Autoclave glucose separately under N<sub>2</sub> at 111°C for 30 min.

### **154. DESULFOVIBRIO SULFODISMUTANS MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

NaCl 1.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g

KCl 0.5 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g

Distilled water 920.0 ml

*Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 2.5 g

Distilled water 50.0 ml

*Solution 4:*

Na-acetate  $\times$  3 H<sub>2</sub>O 0.3 g

Distilled water 10.0 ml

*Solution 5:*

D(+)-Biotin 10.0 mg

Ca-D(+)-Pantothenate 50.0 mg

Distilled water 1.0 ml

*Solution 6:*

Na<sub>2</sub>S  $\times$  9 H<sub>2</sub>O 0.4 g

Distilled water 10.0 ml

*Solution 7:*

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (0.5 M) 10.0 ml

pH 7.5-8.0 (adjust with NaOH)

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub>  $\times$  4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub>  $\times$  4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically at under 80% N<sub>2</sub> + 20% CO<sub>2</sub> at 121°C for 15 min.

Solutions 2, 4, 6 and 7 are gassed with N<sub>2</sub> and autoclaved separately at 121°C for 15 min. Solution 3 (gassed with N<sub>2</sub> + CO<sub>2</sub>) and solution 5 (gassed with N<sub>2</sub>) are filter-sterilized. Solutions with 2 to 7 are added to the sterile, cooled solution 1 in the sequence as indicated. When growth has started feed culture again with same amount of solution 7. After a further two days repeat feeding once more.

### **155. SCHATZ AND BOVELL MEDIUM FOR HYDROGEN-OXIDIZING BACTERIA**

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

NH<sub>4</sub>NO<sub>2</sub> 1.0 g

MgSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.2 g

FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.01 g

CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.01 g

NaHCO<sub>3</sub> 0.5 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 6.8-7.2

Autoclave at 121°C for 15 min. Cultivate in a gas mixture of carbon dioxide, air and hydrogen (1:3:6).

### **156. MEDIUM FOR MYXOBACTERIA**

Casein hydrolysate 2.5 g

Asparagine 2.5 g

K<sub>2</sub>HPO<sub>4</sub> 2.0 g

NaCl 1.0 g

MgSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.1 g

FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.03 g

CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.01 g

Distilled water 1000.0 ml

pH 7.4

Autoclave at 121°C for 15 min.



### **157. EMERSON STARCH YEAST EXTRACT AGAR**

Yeast extract 4.0 g  
Starch (soluble) 15.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

### **158. GLYCEROL YEAST AGAR**

Yeast extract 5.0 g  
Glycerol 50.0 g  
CaCO<sub>3</sub> 1.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2  
Autoclave at 121°C for 15 min.

### **159. SPHAEROTILUS MEDIUM**

Beef extract (Lab Lemco, Oxoid) 5.0 g  
Agar (if necessary) 15.0 g  
Distilled water 1000.0 ml  
Adjust pH to 7.0.  
Prepare sterile agar slants by autoclaving at 121°C for 15 min. Cool the slants in a sloping position. Cover solid slants with 2 ml sterile tap water. Inoculate into the covering tap water.

### **160. MEAT GLUCOSE MEDIUM**

Peptone 10.0 g  
Glucose 10.0 g  
Beef extract 3.0 g  
NaCl 5.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 111°C for 30 min.

### **161. RHIZOBIUM MEDIUM**

Yeast extract 1.0 g  
Mannitol 10.0 g  
Agar 15.0 g  
Soil extract (see below) 200.0 ml  
Distilled water 800.0 ml  
*Soil extract:*  
Air-dried garden soil 80.0 g  
Na<sub>2</sub>CO<sub>3</sub> 0.2 g  
Distilled water 200.0 ml  
Soil extract autoclave at 121°C for 1 h. filter and make up to 200 ml.  
pH 7.2  
Autoclave at 121°C for 15 min.

### **162. ANCYLOBACTER-SPIROSOMA MEDIUM**

Glucose 1.0 g  
Peptone 1.0 g  
Yeast extract 1.0 g

Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2  
Autoclave at 111°C for 30 min.

### **163. MICROCYCLUS MEDIUM**

Glucose 5.0 g  
Peptone 5.0 g  
Yeast extract 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 6.8  
Autoclave at 111°C for 30 min.

### **164. PD BROTH FOR FLEXIBACTER**

Peptone 1.0 g  
KNO<sub>3</sub> 100.0 mg  
Yeast extract 100.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 66.7 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 33.3 mg  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Trace element solution:*  
Zn SO<sub>4</sub> × 7 H<sub>2</sub>O 22.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 1.81 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 79.0 mg  
NaBO<sub>3</sub> × 4 H<sub>2</sub>O 1.0 g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> × 4 H<sub>2</sub>O 9.3 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × H<sub>2</sub>O 20.0 mg  
Trilon B 10.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 111°C for 30 min.

### **165. INDICATOR MEDIUM WITH MALONATE**

Yeast extract 1.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.4 g  
K<sub>2</sub>HPO<sub>4</sub> 0.6 g  
NaCl 2.0 g  
Na-malonate 3.0 g  
Glucose 0.25 g  
Bromothymol blue (0.2%) 12.0 ml  
Distilled water 1000.0 ml  
Dissolve components of the medium in boiling water in the specified sequence (except the indicator). Then filter through a cotton wool-gauze filter to remove the possible precipitate, bring to the initial volume, cool, adjust pH 6.7, add the indicator. Autoclave at 121°C for 15 min.

### **166. CORYNEBACTERIUM AGAR**

Casein peptone, tryptic digest 10.0 g  
Yeast extract 5.0 g  
Glucose 5.0 g  
NaCl 5.0 g

Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 111°C for 30 min.

**167. PEPTONE MEAT AGAR WITH 3% NaCl**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 30.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

**168. CORYNEBACTERIUM MEDIUM WITH 6% NaCl**

Casein peptone, tryptic digest 10.0 g  
Yeast extract 5.0 g  
Glucose 5.0 g  
NaCl 60.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

**169. HETEROTROPHIC MEDIUM H3P**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 2.3 g  
Na<sub>2</sub>HPO<sub>4</sub> × 2 H<sub>2</sub>O 2.9 g  
Distilled water 50.0 ml

*Solution 2:*

NH<sub>4</sub>Cl 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.05 g  
NaVO<sub>3</sub> × H<sub>2</sub>O 0.05 g  
Trace element solution *SL-6* (see below) 5.0 ml  
Agar (if necessary) 15.0 g  
Distilled water 850.0 ml

*Solution 3:*

Fe(NH<sub>4</sub>)-citrate 0.05 g  
Distilled water 20.0 ml

*Solution 4:*

Yeast extract 1.0 g  
Na-acetate × 3 H<sub>2</sub>O 1.0 g  
Na<sub>2</sub>-succinate 1.0 g  
DL-Malate 1.0 g  
Distilled water 30.0 ml  
pH 7.0

*Solution 5:*

Na-lactate 1.0 g  
Na-pyruvate 1.0 g  
D-Mannitol 1.0 g  
D-Glucose 2.0 g  
Distilled water 50.0 ml

pH 7.0

*Trace element solution SL-6:*

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g

H<sub>3</sub>BO<sub>3</sub> 0.3 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g

Na<sub>2</sub>MoO<sub>4</sub> 0.03 g

Distilled water 1000.0 ml

*Vitamin solution:*

Riboflavin 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 50.0 mg

Nicotinic acid 50.0 mg

Pyridoxine-HCl 50.0 mg

Ca-pantothenate 50.0 mg

Biotin 0.1 mg

Folic acid 0.2 mg

Vitamin B<sub>12</sub> 1.0 mg

Distilled water 100.0 ml

Solutions 1, 2, 3, 4 and trace element solution are autoclaved separately for 15 min at 121°C, cooled down to 50°C and then mixed aseptically with filter-sterilized solution 5 (at 50°C) and 5.0 ml of filter-sterilized standard vitamin solution. The final pH of this medium should be 6.8 without adjustment.

### **170. CYTOPHAGA MEDIUM**

Yeast extract 10.0 g

NH<sub>4</sub>Cl 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.2 g

NaCl 20.0 g

FeCl<sub>3</sub> × 6 H<sub>2</sub>O Traces

Agar (if necessary) 2.0-3.0 g

Distilled water 1000.0 ml

pH 7.5

Autoclave at 121°C for 15 min.

### **171. YEAST AGAR**

K<sub>2</sub>HPO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

Yeast extract 10.0 g

Agar 20.0 g

Tap water 1000.0 ml

pH 7.0-7.2

Autoclave at 121°C for 15 min.

### **172. PEPTONE SUCCINATE AGAR**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g

MnSO<sub>4</sub> × 5 H<sub>2</sub>O 2.0 mg

FeCl<sub>3</sub> × 6 H<sub>2</sub>O 2.0 mg

Succinic acid 1.68 g

Peptone 5.0 g

Agar 1.5 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min.

### **173. SPIRILLUM GRACILLE MEDIUM**

Peptone 5.0 g

Yeast extract 0.5 g

Tween 80 0.02 g

K<sub>2</sub>HPO<sub>4</sub> 0.1 g

Agar (if needed) 15.0 g

Tap water 1000.0 ml

pH to 7.2

Autoclave at 121°C for 15 min.

### **174. MEDIUM FOR DENTRIFYING BACTERIA (GILTAY MEDIUM)**

*Solution 1:*

KNO<sub>3</sub> 1.0 g

Asparagine 1.0 g

Distilled water 250.0 ml

*Solution 2:*

Ca-citrate 8.5 g

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

Distilled water 500.0 ml

*Solution 3:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g

FeCl<sub>2</sub> × 4 H<sub>2</sub>O traces

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.2 g

Distilled water 250.0 ml

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

### **175. AQUASPIRILLUM MEDIUM 1**

Peptone 2.0 g

Succinic acid 1.0 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g

Vitamin solution (see below) 1.0 ml

Trace elements (see below) 1.0 ml

Agar (if needed) 15.0 g

Distilled water 1000.0 ml

Adjust pH to 7.4-7.6. Autoclave main medium and trace elements solution separately at 121°C for 15 min and add 1.0 ml/l vitamin solution from a filter sterilized stock solution.

*Trace elements solution (Pfennig & Lippert, 1966):*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.2 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g

H<sub>3</sub>BO<sub>3</sub> 0.03 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g

Distilled water 1000.0 ml

pH 3.0-4.0

*Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml

### **176. METHANOBACTERIUM MEDIUM**

#### *Solution 1:*

NaCl 0.9 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
Yeast extract 2.0 g  
Resazurin 0.01 g  
Trace element solution (see below) 10.0 ml  
Vitamin solution (see below) 5.0 ml  
Buffer solution a (see below) 10.0 ml  
Buffer solution b (see below) 10.0 ml  
Distilled water 965.0 ml

#### *Solution 2 (reducing agents):*

L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

#### *Buffer solutions:*

a) K<sub>2</sub>HPO<sub>4</sub> 29.0 g  
Distilled water 100.0 ml  
b) KH<sub>2</sub>PO<sub>4</sub> 15.0 g  
Distilled water 100.0 ml

#### *Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization. Solutions of reducing agents (10.0 ml) and others solutions add to base medium after separate autoclaving at 121°C for 15 min. Sterilize vitamin solution by filtration.

Cultivate in a gas mixture of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

#### **177. *CYTOPHAGA LYTICA* MEDIUM**

Starch (soluble) 10.0 g  
Yeast extract 1.0 g  
Beef extract 1.0 g  
Pancreatic casein hydrolysate 2.0 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.3

Autoclave at 121°C for 15 min.

#### **178. MEDIUM FOR MARINE *CYTOPHAGA***

*Solution 1:*

Yeast extract 1.0 g  
Tryptone 1.0 g  
KCl 0.7 g  
NaCl 24.7 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 6.3 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.6 g  
Agar 15.0 g  
Distilled water 950.0 ml

*Solution 2:*

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.2 g  
Distilled water 25.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 0.2 g  
Distilled water 25.0 ml

pH 7.2

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

#### **179. *SELENOMONAS RUMINANTIUM* MEDIUM**

Glucose 1.0 g  
Trypticase 5.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
Na-acetate × 3 H<sub>2</sub>O 4.0 g  
Yeast extract 2.0 g  
Valeric acid 0.1 ml  
Resazurin 1.0 mg  
Na<sub>2</sub>CO<sub>3</sub> 4.0 g  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml  
pH 7.0

Autoclave at 111°C for 30 min.

Gas atmosphere: 100% CO<sub>2</sub>.

### 180. *DESULFOVIBRIO CARBINOLICUS* MEDIUM

#### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

#### *Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

#### *Solution 3:*

NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

#### *Solution 4:*

Ethanol 0.7 g  
Casamino acids 0.1 g  
Yeast extract 0.1 g  
Distilled water 10.0 ml

#### *Solution 5:*

Vitamin solution (see below) 10.0 ml

#### *Solution 6:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N<sub>2</sub> at 121°C for 15 min. Vitamin solution is filter sterilized. Others solutions autoclave at 121°C for 15 min and add to base medium aseptically.



Final pH of the complete medium 7.1-7.4

### **181. CASEIN-CITRATE AGAR**

Casein hydrolysate 7.5 g  
Yeast extract 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g  
Na-citrate 3.0 g  
KCl 2.0 g  
NaCl 200.0 g  
4.98% FeSO<sub>4</sub> in 0.01 N HCl 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.4  
Autoclave at 121°C for 15 min.

### **182. DESULFOBACTERIUM MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 21.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 3.0 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
Resazurin 1.0 mg  
Distilled water 930.0 ml

*Solution 2:*

Trace element solution *SL-10* (see below) 1.0 ml

*Solution 3:*

Vitamin solution (see below) 10.0 ml

*Solution 4:*

NaHCO<sub>3</sub> 2.5 g  
Distilled water 50.0 ml

*Solution 5:*

Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O (3 mg in 1000.0 ml 0.01 M NaOH) 1.0 ml

*Solution 6:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

*Solution 7:*

Substrate - depending on the species of bacteria:

25% Na-acetate 10.0 ml or indole 0.3 g

NaCl 2.1 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.3 g

Distilled water 100.0 ml

Phenol 40.0 mg

or Na-benzoate 400.0 mg

Distilled water 4.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

*p*-Aminobenzoic acid 4.0 mg  
D(+)-Biotin 1.0 mg  
Thiamine-HCl 10.0 mg  
Distilled water 100.0 ml  
pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> at 121°C for 15 min. Solutions 2, 4, 5 and 6 are gassed with N<sub>2</sub> and autoclaved separately at 121°C for 15 min. Solution 3 (gassed with N<sub>2</sub> + CO<sub>2</sub>) and solution 7 (gassed with N<sub>2</sub>) are filter-sterilized.

**183. BENETT MEDIUM**

Yeast extract 1.0 g  
Meat extract 1.0 g  
Fermentative casein hydrolysate 2.0 g  
Glucose 10.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 111°C for 30 min.

**184. ISP 2 MEDIUM**

Glucose 4.0 g  
Yeast extract 4.0 g  
Malt extract 10.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 111°C for 30 min.

**185. HALOBACTERIUM MEDIUM 4**

Yeast extract 5.0 g  
Casamino acids 5.0 g  
Na-glutamate 1.0 g  
KCl 2.0 g  
Na-citrate 3.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g  
NaCl 200.0 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 36.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.36 mg  
Agar 20.0 g  
Distilled water to 1000.0 ml  
pH 7.0-7.2  
Autoclave at 111°C for 30 min.

**186. HALOBACTERIUM MEDIUM 5**

*Solution 1:*

Casamino acids 7.5 g  
Yeast extract 10.0 g  
Na-citrate 3.0 g  
KCl 2.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.05 g

MnSO<sub>4</sub> × H<sub>2</sub>O 0.2 g

NaCl 250.0 g

Distilled water 750.0 ml

*Solution 2:*

Agar 20.0 g

Distilled water 250.0 ml

pH 7.4

Autoclave separately at 121°C for 15 min and mix aseptically.

### **187. HALOCOCCUS MEDIUM**

*Solution 1:*

Skim milk 50.0 g

Distilled water 500.0 ml

*Solution 2:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 g

KNO<sub>3</sub> 2.0 g

NaCl 200.0 g

Fe-citrate traces

Distilled water 100.0 ml

*Solution 3:*

Neopeptone 5.0 g

Glycerol 10.0 g

Agar 25.0 g

Distilled water 400.0 ml

Autoclave of solution 1 at 111°C for 15 min. Mix together heated solutions 2 and 3, adjust pH of the mixture to 8.4 and autoclave at 121°C for 15 min.

### **188. NATRONOBACTERIUM MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

KCl 1.0 g

NH<sub>4</sub>Cl 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.24 g

CaSO<sub>4</sub> × 2 H<sub>2</sub>O 0.17 g

Trace element solution *SL-10* (see below) 1.0 ml

NaCl 200.0 g

Glutamate 1.0 g

Yeast extract 5.0 g

Casamino acids 5.0 g

Agar, if necessary (heat and dissolve it before adding sodium chloride) 20.0 g

Distilled water to 1000.0 ml

pH 6.5

*Solution 2:*

Na<sub>2</sub>CO<sub>3</sub> 5.0 g

Distilled water 50.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
pH 9.0-9.5  
Autoclave separately at 121°C for 15 min and mix aseptically.

### **189. HALOBACTERIUM MEDIUM 6**

NaCl 156.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 13.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 g  
KCl 4.0 g  
NaHCO<sub>3</sub> 0.2 g  
NaBr 0.5 g  
Yeast extract 5.0 g  
Glucose 1.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 111°C for 30 min.

### **190. PRAUSER MEDIUM 79**

Glucose 10.0 g  
Peptone 10.0 g  
Yeast extract 2.0 g  
Casamino acids 2.0 g  
NaCl 6.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.5  
Autoclave at 111°C for 30 min.

### **191. STARCH-YEAST AGAR**

Yeast extract 2.0 g  
Starch (soluble) 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.3  
Autoclave at 121°C for 15 min.

### **192. MYA-AGAR**

Glucose 2.0 g  
L-Asparagine 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Trace element solution (see below) 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
*Trace element solution:*  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Distilled water 100.0 ml  
pH 7.4

Autoclave base medium at 111°C for 30 min, trace element solution at 121°C for 15 min.

### **193. ACETATE AGAR**

NaCl 5.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
Na-acetate × 3 H<sub>2</sub>O 2.0 g  
Bromothymol blue (0.2%) 40.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2

Add the indicator last, after pH is set and the possible precipitate is separated by filtration through a cotton wool-gauze filter.

Autoclave at 121°C for 15 min.

### **194. INMI MEDIUM 3**

NaCl 250.0 g  
KCl 2.0 g  
Na-citrate 3.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g  
Casamino acids 5.0 g  
Yeast extract 2.5 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2

Autoclave at 121°C for 15 min.

### **195. INMI MEDIUM 4**

Yeast extract 2.5 g  
Casamino acids 5.0 g  
pH 9.5

Autoclave at 121°C for 15 min.

### **196. MEDIUM FOR PURPLE BACTERIA (VAN NIEL MEDIUM)**

NH<sub>4</sub>Cl 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
NaHCO<sub>3</sub> 1.0 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g  
Tap water 1000.0 ml  
pH 7.6

Autoclave base medium and sulfide separately at 121°C for 15 min.

### **197. MEDIUM FOR *RHODOSPIRILLUM* (PFENNIG MEDIUM)**

NH<sub>4</sub>Cl 0.4 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
NaCl 0.4 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
Acetate, or butyrate, or propionate, or succinate 1.0 g  
Yeast extract 0.2 g  
Fe-citrate (0.1%) 5.0 ml  
Trace element solution according to *Pfennig* (see below) 1.0 ml

Vitamin B<sub>12</sub> (dispensary solution, 0,01%) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution according to Pfennig:*

EDTA 1.5 g

Trace element solution according to *Hogland* (see below) 6.0 ml

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

Autoclave base medium and trace element solution separately at 121°C for 15 min.

#### **198. THIOBACILLUS DENITRIFICANS MEDIUM (TAYLOR MEDIUM)**

KNO<sub>3</sub> 2.0 g

NH<sub>4</sub>Cl 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 2.0 g

NaHCO<sub>3</sub> 2.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.8 g

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 5.0 g

Trace element solution according to *Pfennig* (see below) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution according to Pfennig:*

EDTA 1.5 g

Trace element solution according to *Hogland* (see below) 6.0 ml

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

Autoclave base medium and trace element solution separately at 121°C for 15 min.

#### **199. THIOBACILLUS DENITRIFICANS MEDIUM (BAALSRUD MEDIUM)**

KNO<sub>3</sub> 2.0 g

NH<sub>4</sub>Cl 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
NaHCO<sub>3</sub> 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 5.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
Distilled water 1000.0 ml  
pH 7.0

Autoclave stock solutions of iron, phosphorus and bicarbonate salts separately at 121°C for 15 min.

#### **200. THIOBACILLUS DENITRIFICANS MEDIUM (LIESKE MEDIUM)**

KNO<sub>3</sub> 5.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NaHCO<sub>3</sub> 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 5.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O Traces  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O Traces  
Distilled water 1000.0 ml  
Autoclave at 121°C for 15 min.

#### **201. MODIFIED BROCK MEDIUM FOR SULFUROXIDIZING BACTERIA**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.3 g  
KH<sub>2</sub>PO<sub>4</sub> 0.37 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.25 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.07 g  
Distilled water 1000.0 ml  
pH 2.4 (range: 2.3-3.0; adjust with H<sub>2</sub>SO<sub>4</sub>).

Additions to the medium

Trace element solution according to *Pfennig* (see below) 1.0 ml

Yeast extract 0.2 g

Element sulfur 10.0 g

Chalk (CaCO<sub>3</sub>) 10.0 g

For cultivation of heterotrophic representatives of the group the medium after sterilization is to be also supplemented with

Peptone 1.75 g

Sucrose 0.25 g

*Trace element solution according to Pfennig:*

EDTA 1.5 g

Trace element solution according to *Hogland* (see below) 6.0 ml

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

Autoclave base medium and others components of medium separately at 121°C for 15 min.

## **202. MEDIUM FOR MAGNETIC BACTERIA**

Tartaric acid 0.37 g  
Succinic acid 0.37 g  
Na-acetate × 3 H<sub>2</sub>O 0.05 g  
KH<sub>2</sub>PO<sub>4</sub> 0.68 g  
NaNO<sub>3</sub> 0.12 g  
Vitamin solution (see below) 10.0 ml  
Trace element solution (see below) 5.0 ml  
Fe-quininate 2.0 ml  
Na-thioglycolate 0.05 g  
Resazurin 0.5 mg  
Agar 1.3 g  
Bidistilled water 1000.0 ml

### *Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml

### *Trace element solution:*

Nitrilotriacetic acid 12.8 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.17 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnCl<sub>2</sub> 0.1 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.026 g  
NaCl 1.0 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml

pH 6.75 (adjust with NaOH)

Prior to sterilization the medium is to be blown down by the flow of N<sub>2</sub> and autoclaved in the nitrogen atmosphere at 121°C for 15 min. Vitamin solution is filter sterilized. Trace element solution autoclave at 121°C for 15 min and add to base medium aseptically.

## **203. MEDIUM FOR *FLEXIBACTER* (LEWIN MEDIUM)**

Na-glycerophosphate 0.1 g  
KNO<sub>3</sub> 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Trace element solution (see below) 1.0 ml  
Vitamin B<sub>12</sub> 1.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
Tris 1.0 g  
Thiamine 1.0 mg  
Casamino acids 1.0 g



Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 g

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.17 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

ZnCl<sub>2</sub> 0.1 g

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g

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

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.026 g

NaCl 1.0 g

Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

pH 7.5

Autoclave base medium and trace element solution separately at 121°C for 15 min.

#### **204. MEDIUM FOR *CLOSTRIDIUM***

Glucose 10.0 g

Peptone 12.0 g

NaCl 2.0 g

Agar 16.0 g

Distilled water 1000.0 ml

The medium can be used with chalk addition as buffer against acidification of the medium during cultivation.

pH to 6.8-7.2

Autoclave at 111°C for 30 min.

#### **205. OATMEAL AGAR A**

Oatmeal or oat flakes 20.0 g

Agar 20.0 g

Tap water 1000.0 ml

pH to 7.2

Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.

Autoclave at 121°C 15 min.

#### **206. ISP MEDIUM 3**

Oatmeal 20.0 g

Salt solution A (see below) 1.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

*Salt solution A:*

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

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Distilled water 100.0 ml

Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.

pH 7.2

Autoclave separately at 121°C 15 min.

#### **207. MINERAL AGAR 1**

Starch (soluble) 20.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

KNO<sub>3</sub> 1.0 g  
NaCl 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
Agar 30.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C 15 min.

#### **208. GLUCOSE ASPARAGINE AGAR**

Glucose 10.0 g  
L-Asparagine 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 6.8.  
Autoclave at 111°C for 30 min.

#### **209. MODIFICATION OF THE CZAPEK MEDIUM WITH STARCH**

Starch (soluble) 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
KNO<sub>3</sub> 1.0 g  
NaCl 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.3 g  
CaCO<sub>3</sub> 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C 15 min.

#### **210. GLYCEROL-ASPARAGINE AGAR**

L-Asparagine 1.0 g  
Glycerol 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
Salt solution A (see below) 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
*Salt solution A:*  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Distilled water 100.0 ml  
pH 7.0-7.4  
Autoclave separately at 121°C 15 min.

#### **211. OATMEAL AGAR WITH 0.1% YEAST EXTRACT**

Oatmeal 20.0 g  
Yeast extract 1.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.2  
Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.  
Autoclave at 121°C 15 min.

## **212. MODIFICATION OF CZAPEK MEDIUM WITH GLUCOSE**

Glucose 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
KNO<sub>3</sub> 1.0 g  
NaCl 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.3 g  
CaCO<sub>3</sub> 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Autoclave at 111°C for 30 min.

## **213. ORGANIC AGAR 2**

Hottinger broth (HiMedia M1425) 0.69 g  
Peptone 5.0 g  
NaCl 5.0 g  
Glucose 10.0 g  
Agar 30.0 g  
Tap water 1000.0 ml  
pH 7.0-7.2  
Autoclave at 111°C for 30 min.

## **214. STARCH AMMONIA AGAR**

Starch (soluble) 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
NaCl 1.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
CaCO<sub>3</sub> 2.0 g  
Salt solution A (see below) 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
*Salt solution A:*  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Distilled water 100.0 ml  
pH 7.0-7.4  
Autoclave separately at 121°C 15 min.

## **215. GLYCEROL NITRATE AGAR**

Glycerol 30.0 g  
NaNO<sub>3</sub> 2.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
KCl 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2.  
Autoclave at 121°C for 15 min.

## **216. TETRATHIONATE BROTH (MULLER MEDIUM)**

*Solution 1:*

Hottinger broth (HiMedia M1425) 20.24 g

Distilled water 880.0 ml

*Solution 2:*

Lugol solution (see below) 20.0 ml

*Solution 3:*

50% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 100.0 ml

*Lugol solution:*

KI 20.0 g

I<sub>2</sub> 25.0 g

Distilled water 100.0 ml

pH 7.2 - 7.4

Autoclave solution 3 at 121°C 15 min. Lugol solution is filter sterilized. Pour the medium into sterile vials with CaCO<sub>3</sub> (25 g CaCO<sub>3</sub> per 1000.0 ml of medium). Sterilize the flasks with CaCO<sub>3</sub> with dry heat (170 C for 60 min).

### **217. MEDIUM FOR MICROMONOSPORES**

Glucose 10.0 g

Starch (soluble) 20.0 g

Yeast extract 5.0 g

Fermentative casein hydrolysate 5.0 g

CaCO<sub>3</sub> 1.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 111°C for 30 min.

### **218. PYA WITH MARINE WATER**

Peptone 5.0 g

Yeast extract 3.0 g

Agar 12.0 g

Distilled water 250.0 ml

Aged filtered sea water 750.0 ml

pH 7.5-7.6

Autoclave at 121°C 15 min.

### **219. MEDIUM FOR HALOPHILIC BACILLI**

NaCl 100.0 g

NaHCO<sub>3</sub> 10.0 g

Na<sub>2</sub>CO<sub>3</sub> 10.0 g

Nutrient broth 1000.0 ml

pH 9.5

Autoclave at 121°C 15 min.

### **220. PYEA MEDIUM**

Peptone 10.0 g

Yeast extract 10.0 g

NaCl 5.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 121°C 15 min.

### **221. MEDIUM FOR METHANOTROPHIC BACTERIA**

KNO<sub>3</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.7 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 1.5 g  
EDTA 5.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 mg  
Distilled water 1000.0 ml  
pH 6.7-7.1  
Autoclave at 121°C 15 min.  
Cultivation under mixture of methane and air (1:1).

## **222. MEDIUM FOR MARINE METHYLOTROPHIC BACTERIA**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
NaCl 30.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Yeast extract 0.1 g  
Methanol 5.0 ml  
Biotin 0.01 mg  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C 15 min. After autoclaving add filter sterilize methanol and biotin.

## **223. MEDIUM FOR OLIGOCARBOPHILIC BACTERIA**

*Solution 1 (basic solution):*

Peptone 0.25 g  
Yeast extract 0.25 g  
Agar 5.0 g  
Solution 2 20.0 ml  
Distilled water 965.0 ml  
Glucose (2.5 %) 10.0 ml  
Solution 3 5.0 ml  
pH 7.5

*Solution 2 (trace elements):*

Nitilotriacetic acid 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 350.0 mg  
Na-EDTA 125.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 548.0 mg  
MnSO<sub>4</sub> × H<sub>2</sub>O 77.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 20.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 12.4 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 8.8 mg  
Distilled water 950.0 ml

Dissolve nitrilotriacetic acid first by neutralizing with KOH, then add other salts. Adjust volume to 1000.0 ml.

Adjust pH to 7.2

*Solution 3 (vitamin solution):*

Biotin 4.0 mg

Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg

Riboflavine 10.0 mg

Thiamine-HCl 10.0 mg

Nicotin amide 10.0 mg

Ca-D-pantothenate 10.0 mg

Vitamin B<sub>12</sub> 0.2 mg

*p*-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

pH 6.8–7.0

Autoclave glucose stock solution at 111°C for 30 min, solutions 1 and 2 at 121°C 15 min. Vitamin solution is filter sterilized. Store in refrigerator at +5 C.

## **224. MEDIUM FOR *DESULFOTOMACULUM ALKALIPHILUM***

*Solution 1 (basic solution):*

Na<sub>2</sub>CO<sub>3</sub> 0.5 g

Na<sub>2</sub>SO<sub>4</sub> 5.0 g

NaCl 5.0 g

Na formate 5.0 g

Yeast extract 1.0 g

Solution 2 (see below) 10.0 ml

Solution 3 (see below) 2.0 ml

Solution 4 (see below) 1.0 ml

Rezazurine traces

Distilled water 1000.0 ml

NaHCO<sub>3</sub> final concentration 8.0 g /1000.0ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O final concentration 0.5 g/1000.0 ml

*Solution 2:*

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g

NH<sub>4</sub>Cl 1.0 g

KCl 0.2 g

Distilled water 1000.0 ml

*Solution 3 (vitamin solution):*

Biotin 10.0 mg

Folic acid 10.0 mg

Pyridoxine-HCl 50.0 mg

Riboflavine 25.0 mg

Thiamine-HBr 25.0 mg

Nicotin amide 25.0 mg

D-pantothenate 25.0 mg

Vitamin B<sub>12</sub> 0.5 mg

*p*-Aminobenzoic acid 25.0 mg

Thioctic acid 25.0 mg

Distilled water 500.0 ml

Store in refrigerator at +5°C.

*Solution 4 (trace elements):*

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg

Fe (NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 400.0 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 200.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 200.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 720.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 100.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 20.0 mg  
AlK(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 20.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg  
H<sub>3</sub>BO<sub>3</sub> 20.0 mg  
HCl 5.0 ml

Distilled water 200.0 ml

pH of the autoclaved medium is 8.7-9.0

Vitamin solution is filter sterilized. Others solutions autoclave separately at 121°C for 15 min and add to the Solution 1 after cooling.

### **225. 1/5 STARCH-YEAST AGAR**

Yeast extract 0.4 g

Soluble starch 2.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.3

Autoclave at 121°C 15 min.

### **226. MEDIUM FOR *ACTINOPOLYSPORA MORTIVALLIS***

Bacto vitamin assay casamino acids 7.5 g

Yeast extract 10.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g

Trisodium citrate × 2 H<sub>2</sub>O 3.0 g

KCl 2.0 g

NaCl 150.0 g

4.98% FeSO<sub>4</sub> in 0.01 N HCl 1.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.4

Autoclave at 121°C 15 min.

### **227. *MICROLUNATUS* MEDIUM**

Glucose 0.5 g

Peptone 0.5 g

Yeast extract 0.5 g

Na- glutamate 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.44 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min.

### **228. *MICROCOCCUS HALOPHILUS* MEDIUM**

Peptone 10.0 g

Yeast extract 5.0 g

Malt extract 5.0 g

Casamino acids 5.0 g

Meat extract 2.0 g

Glycerol 2.0 g

Tween-80 50.0 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
NaCl 50.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 121°C 15 min.

### **229. ALKALIBACTER MEDIUM**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
NH<sub>4</sub>Cl 0.4 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 0.1 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
NaCl 10.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 mg  
Trace element solution (see below) 10.0 ml  
Resazurin 0.01 g  
Yeast extract 0.25 g  
Tryptone 2.0 g  
Glucose 5.0 g  
Vitamin solution (see below) 10.0 ml  
L-cysteine 0.5 g  
Na<sub>2</sub>CO<sub>3</sub> (5% w/v) 50.0 ml  
Distilled water 930.0 ml

#### *Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Basal medium autoclave at 111°C for 30 min. Vitamin solution is filter sterilized. Others solutions autoclave at 121°C for 15 min (Na<sub>2</sub>CO<sub>3</sub> is autoclaved under 100% N<sub>2</sub>).

Final pH 9.0



### **230. CALDITHRIX ABYSSI MEDIUM**

Sea salt 37.9 g  
NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Resazurin 0.5 mg  
Yeast extract (20% w/v) 15.0 ml  
Trace element solution (see below) 10.0 ml  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O (3% w/v) 20.0 ml  
Selenite-tungstate solution (see below) 1.0 ml

Distilled water 900.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 mg

NaCl 1.0 mg

Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g

Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg

Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg

Distilled water 1000.0 ml

Prepare medium anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. After autoclaving add from separately prepared, sterile anaerobic stock solutions NaHCO<sub>3</sub> (5% w/v) 50.0 ml. Vitamin solution is filter sterilized. Others solutions autoclave separately at 121°C for 15 min.

Final pH of the medium to 6.8

### **231. CARBOXYDOCELLA SPOROPRODUCENS MEDIUM**

KCl 0.33 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.52 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.29 g

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

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

NaHCO<sub>3</sub> 1.0 g

Trace element solution *SL-4* (see below) 10.0 ml  
Resazurin 0.5 mg  
Vitamin solution (see below) 10.0 ml  
Pyruvate 2.5 g  
Yeast extract 0.05 g  
 $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.3 g  
Distilled water 1000.0 ml  
*Trace element solution SL-4:*  
EDTA 0.5 g  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.2 g  
Trace element solution *SL-6* (see below) 100.0 ml  
Distilled water 900.0 ml

*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Trace element solution SL-6:*  
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  0.03 g  
 $\text{H}_3\text{BO}_3$  0.3 g  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  0.2 g  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  0.01 g  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.02 g  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  0.03 g  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, yeast extract, vitamins, pyruvate and sulfide, boil medium for 1 min, then cool to room temperature under N<sub>2</sub> gas atmosphere. Dispense medium under same gas atmosphere in tubes or serum bottles and autoclave at 121°C for 15 min. Add vitamins (sterilized by filtration), yeast extract, pyruvate and sulfide from sterile anoxic stock solutions prepared under N<sub>2</sub> gas atmosphere and bicarbonate from a sterile anoxic solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture (all solutions autoclave separately at 121°C 15 min). Adjust pH to 7.0 with a sterile anoxic solution of 10% (w/v) NaHCO<sub>3</sub>. Inoculated vessels are pressurized with carbon monoxide gas to 2 bar overpressure.

### **232. CARBOXYDOTHERMUS FERRIREDUCENS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.33 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.33 g  
NaHCO<sub>3</sub> 2.0 g  
Glycerol (87%) 3.0 ml  
Vitamin solution (see below) 10.0 ml  
Trace element solution *SL-10* (see below) 1.0 ml  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  200.0 µg  
 $\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  120.0 µg

Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 30.0 µg  
Yeast extract 1.0 g  
Na<sub>2</sub>-9,10-anthraquinone-2,6-disulfonate (Sigma A9706) 8.25 g  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

Dissolve ingredients (except CaCl<sub>2</sub> × 2 H<sub>2</sub>O, NaHCO<sub>3</sub>, and vitamins), boil medium for some minutes to dissolve the anthraquinone, then cool under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere to room temperature. Add solid NaHCO<sub>3</sub> and adjust medium pH to 6.8 with NaOH. Dispense medium in tubes or bottles under same gas. Autoclave at 121°C for 15 min. Before use, add CaCl<sub>2</sub> × 2 H<sub>2</sub>O and vitamins from anoxic, sterile stock solutions. Vitamin solution is filter sterilized. CaCl<sub>2</sub> × 2 H<sub>2</sub>O and trace element solution Autoclave at 121°C for 15 min

### **233. CLOSTRIDIUM ALKALICELLULOSI MEDIUM**

NH<sub>4</sub>Cl 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
KCl 0.2 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
Na<sub>2</sub>CO<sub>3</sub> 1.0 g  
NaHCO<sub>3</sub> 7.6 g  
NaCl 10.0 g  
Yeast extract 0.2 g  
Cellobiose 3.0 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

Dissolve ingredients except cellobiose and sulfide, flush medium with N<sub>2</sub> gas for 30–60 min, dispense under N<sub>2</sub> gas atmosphere and autoclave at 121°C 15 min. Add cellobiose after autoclaving from an anoxic stock solution sterilized by filtration and sulfide from a sterile (Autoclave at 121°C 15 min), anoxic stock solution prepared under N<sub>2</sub>.

Final pH of the medium 8.8-9.0.

**234. DESULFOHALOBIUM UTAHENSE MEDIUM**

NaCl 100.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 g  
KCl 6.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.4 g  
NH<sub>4</sub>Cl 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
Yeast extract 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml

NaHCO<sub>3</sub> 4.0 g  
Na-(L)-lactate 2.5 g  
Resazurin 0.5 mg  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.3 g

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except lactate, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Dispense under same gas atmosphere in culture vessels and autoclave at 121°C 15 min. Add sodium lactate and sulfide from sterile anoxic stock solutions prepared under N<sub>2</sub> and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> (all solutions sterilize separately at 121°C 15 min).

Final pH of the medium 7.0-7.2

**235. DESULFONATRONUM COOPERATIVUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
KCl 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 5.0 g  
NaCl 10.0 g  
Na<sub>2</sub>CO<sub>3</sub> 3.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Vitamin solution (see below) 10.0 ml

Yeast extract 1.0 g  
Resazurin 0.5 mg  
Na-formate 4.0 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve the ingredients (except formate, vitamin solution and sulfide) and flush medium with 100 % N<sub>2</sub> for 30 min. Add the sodium sulfide, adjust the pH to 8.8-9.0, dispense in Hungate tubes under N<sub>2</sub>, and autoclave at 121°C 15 min. Before use add sodium formate and vitamin solution from a sterile, anaerobic stock solution (autoclave sodium formate 121°C 15 min and vitamin solution by filtration).

**236. *DESULFOTOMACULUM CARBOXYDIVORANS* MEDIUM**

*Solution 1:*

NaCl 1.17 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KCl 0.3 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
NH<sub>4</sub>Cl 0.27 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 2.84 g  
Na-pyruvate 2.2 g  
Yeast extract 0.5 g  
Vitamin solution (see below) 1.0 ml

Trace element solution 1.0 ml (see below)

Distilled water 940.0 ml

*Solution 2:*

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.36 g

Distilled water 10.0 ml

*Sodium bicarbonate for alkalization:*

$\text{NaHCO}_3$  4.5 g

Distilled water 50.0 g

*Vitamin solution:*

*p*-Aminobenzoic acid 4.0 mg

D(+)-Biotin 1.0 mg

Thiamine-HCl 10.0 mg

Distilled water 100.0 ml

*Trace element solution:*

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.5 g

$\text{ZnCl}_2$  68.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg

$\text{H}_3\text{BO}_3$  62.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  120.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  24.0 mg

HCl (0.05 M) 1000.0 ml

pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80%  $\text{N}_2$  and 20%  $\text{CO}_2$  with sodium bicarbonate added until an equilibrium pH of 6.9 - 7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100%  $\text{N}_2$  at 121°C for 15 min. Vitamin solution and Na-pyruvate is filter sterilized.

### **237. *DESULFUROCOCCUS FERMENTANS* MEDIUM**

$\text{NH}_4\text{Cl}$  0.33 g

$\text{KH}_2\text{PO}_4$  0.33 g

KCl 0.33 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.44 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.7 g

NaCl 0.5 g

Trace elements *SL-10* (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Yeast extract 0.2 g

Starch 5.0 g

Resazurin 1.0 mg

$\text{NaHCO}_3$  0.8 g

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.5 g

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.5 g

$\text{ZnCl}_2$  70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg

$\text{H}_3\text{BO}_3$  6.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except vitamins, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust pH to 6.2-6.4 and autoclave at 121°C 15 min. After autoclaving add vitamins from an anoxic stock solution sterilized by filtration and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture (autoclave at 121°C 15 min). Prior to inoculation reduce medium by adding sulfide from a sterile, anoxic stock solution prepared under N<sub>2</sub> (autoclave at 121°C 15 min).

### **238. AQUASPIRILLUM MEDIUM II**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 30.0 mg  
Na<sub>2</sub>HPO<sub>4</sub> 10.0 mg  
Casamino acids 1.5 g  
Sodium succinate (10% solution) 10.0 ml  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O (10% solution) 1.0 ml  
Vitamin solution (see below) 5.0 ml  
Trace element solution *SL-10* (see below) 1.0 ml

#### *Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
Agar 0.5 g  
Distilled water 985.0 ml  
pH 7.5

Basal medium, trace element solution autoclave separately at 121°C 15 min. Vitamin solution sterilize by filtration.

### **239. METHYLOPHAGA ALCALICA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

KNO<sub>3</sub> 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.22 g

NaCl 30.0 g

Na<sub>2</sub>CO<sub>3</sub> 5.0 g

Trace element solution (see below) 1.0 ml

Vitamin B<sub>12</sub> 0.02 mg

Methanol 10.0 ml

Distilled water 1000.0 ml

Final pH 9.5

*Trace elements solution:*

Ferric citrate 30.0 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 30.0 mg

MgCl<sub>2</sub> × 4 H<sub>2</sub>O 5.0 mg

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 5.0 mg

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.5 g

Distilled water 1000.0 ml

Prepare the medium without the Na<sub>2</sub>CO<sub>3</sub>, vitamin B<sub>12</sub> and methanol. Basal medium, trace element and Na<sub>2</sub>CO<sub>3</sub> autoclave separately at 121°C 15 min. Add the vitamin B<sub>12</sub> solution from a filter-sterilized stock solution and filter-sterilized methanol to the cooled medium. When preparing liquid media cool the mineral salts solution and Na<sub>2</sub>CO<sub>3</sub> to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool the Na<sub>2</sub>CO<sub>3</sub> stock solution and agar to 50-55°C before mixing.

### **240. METHYLOTHERMUS THERMALIS MEDIUM**

KNO<sub>3</sub> 0.25 g

NH<sub>4</sub>Cl 0.25 g

KH<sub>2</sub>PO<sub>4</sub> 0.13 g

Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 0.358 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 6.8

The gas phase methane/air mixture (4:1)

Autoclave at 121°C 15 min.

### **241. OCEANITHERMUS PROFUNDUS MEDIUM**

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

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g

KCl 0.33 g

KNO<sub>3</sub> 0.33 g

NaCl 30.0 g

HEPES 2.38 g

Yeast extract 0.2 g

Tryptone 1.0 g

Sucrose 2.0 g

Vitamin solution (see below) 1.0 ml

Trace elements (see below) 1.0 ml



Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 10.0 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

*p*-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

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

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg

CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg

ZnCl<sub>2</sub> 0.1 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg

H<sub>3</sub>BO<sub>3</sub> 0.01 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 mg

NaCl 1.0 mg

Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg

Distilled water 1000.0 ml

Prepare the medium anaerobically, under N<sub>2</sub>, omitting the CaCl<sub>2</sub> × 2 H<sub>2</sub>O, MgCl<sub>2</sub> × 6 H<sub>2</sub>O, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The pH should be 7.0-7.5. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of N<sub>2</sub> and autoclave at 121°C 15 min. To the sterile, cooled medium add, from sterile stock solutions the CaCl<sub>2</sub> × 2 H<sub>2</sub>O, MgCl<sub>2</sub> × 6 H<sub>2</sub>O, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The CaCl<sub>2</sub> × 2 H<sub>2</sub>O, MgCl<sub>2</sub> × 6 H<sub>2</sub>O, KNO<sub>3</sub>, tryptone, and yeast extract, stock solutions should be autoclaved at 121°C 15 min, sucrose at 111° 30 min, while the vitamin solution is sterile filtered.

## **242. PSYCHROBACTER MEDIUM**

Peptone 5.0 g

Yeast extract 1.0 g

Sea salts 17.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 121°C 15 min.

## **243. ROSEICYCLUS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.3 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

NH<sub>4</sub>Cl 0.3 g

KCl 0.3 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g

Na<sub>2</sub>SO<sub>4</sub> 15.0 g

NaHCO<sub>3</sub> 0.5 g

Na-acetate × 3 H<sub>2</sub>O 1.0 g

Na-malate 1.0 g

Yeast extract 1.0 g

Peptone 0.5 g

Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.8-8.0  
Autoclave at 121°C 15 min.

#### **244. SULFOBACILLUS MEDIUM**

##### *Solution A:*

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 3.0 g  
KCl 0.1 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
Ca(NO<sub>3</sub>)<sub>2</sub> 0.01 g  
Distilled water 680.0 ml  
pH 2.0-2.2 (adjust with sulfuric acid)

##### *Solution B:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 44.2 g  
Distilled water 300.0 ml  
H<sub>2</sub>SO<sub>4</sub> (10 N) 1.0 ml

##### *Solution C:*

Yeast extract (1% w/v in water) 20.0 ml  
pH 1.9-2.4

After autoclaving at 121°C 15 min, combine the three solutions.

#### **245. THERMINCOLA MEDIUM**

NH<sub>4</sub>Cl 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
KCl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
Resazurin 0.5 mg  
*Wolfe's* mineral elixir (see below) 1.0 ml  
Vitamin solution (see below) 20.0 ml  
NaHCO<sub>3</sub> 0.5 g  
Na<sub>2</sub>CO<sub>3</sub> 0.5 g  
Na-acetate × 3 H<sub>2</sub>O 0.2 g  
Yeast extract 0.2 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g  
Distilled water 1000.0 ml  
*Wolfe's mineral elixir:*  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 30.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 5.0 g  
NaCl 10.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 1.8 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1.8 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.1 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.18 g  
H<sub>3</sub>BO<sub>3</sub> 0.1 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 2.8 g  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 g  
Na<sub>2</sub>SeO<sub>4</sub> 0.1 g  
Distilled water 1000.0 ml

First adjust pH to 1.0 with diluted H<sub>2</sub>SO<sub>4</sub>, then add and dissolve the salts.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except carbonates, vitamin solution, yeast extract and sulfide, boil medium for 1 min, then cool to room temperature under N<sub>2</sub> gas atmosphere. Add carbonates and sulfide to the medium, dispense under CO gas atmosphere in culture vessels (e.g., 10.0 ml medium in 50 ml serum bottles) and autoclave at 121°C 15 min. Prior to inoculation add yeast extract from a sterile, anoxic stock solution prepared under N<sub>2</sub> and adjust pH of final medium to 8.0 with a sterile, anoxic solution of 1 N HCl (this solutions autoclave at 121°C 15 min). Add filter-sterilized vitamin solution.

**246. VULCANITHERMUS MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
KCl 0.33 g  
KNO<sub>3</sub> 0.33 g  
NaCl 25.0 g  
PIPES 3.6 g  
Yeast extract 0.5 g  
Tryptone 1.0 g  
Sucrose 1.0 g  
Vitamin solution (see below) 1.0 ml  
Trace elements solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg

H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml  
pH 6.8

Prepare the medium anaerobically, under N<sub>2</sub>, omitting the CaCl<sub>2</sub> × 2 H<sub>2</sub>O, MgCl<sub>2</sub> × 6 H<sub>2</sub>O, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of N<sub>2</sub> and autoclave at 121°C 15 min. To the sterile, cooled medium add, from sterile stock solutions the CaCl<sub>2</sub> × 2 H<sub>2</sub>O, MgCl<sub>2</sub> × 6 H<sub>2</sub>O, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The CaCl<sub>2</sub> × 2 H<sub>2</sub>O, MgCl<sub>2</sub> × 6 H<sub>2</sub>O, KNO<sub>3</sub>, tryptone, and yeast extract, stock solutions should be autoclaved at 121°C 15 min, sucrose at 111°C 30 min, while the vitamin solution is sterile filtered.

#### **247. RHODOBLASTUS MEDIUM**

Yeast extract 0.1 g  
Na<sub>2</sub>-succinate 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g  
NaCl 0.4 g  
NH<sub>4</sub>Cl 0.4 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
Trace element solution *SL-6* (see below) 1.0 ml  
Methanol 0.01-1%  
Distilled water 1000.0 ml  
pH 5.7

*Trace element solution SL-6:*

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

Autoclave basal medium and trace element solution separately at 121°C 15 min, methanol sterilize by filtration.

#### **248. PFENNIG'S MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.34 g  
NH<sub>4</sub>Cl 0.34 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
KCl 0.34 g  
Trace element solution *SLA* (see below) 1.0 ml  
Vitamin B<sub>12</sub> 20 µg  
NaHCO<sub>3</sub> 1.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.4 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 0.5 g  
NaCl 15.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 2.5 g  
pH 7.5

*Trace element solution SLA:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.8 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 250 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 10 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 70 mg  
ZnCl<sub>2</sub> 100 mg  
H<sub>3</sub>BO<sub>3</sub> 500 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 30 mg  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 10 mg  
Distilled water 1000.0 ml  
Autoclave basal medium and trace element solution separately at 121°C 15 min, vitamin B<sub>12</sub> sterilize by filtration.

#### **249. METHYLOPHAGA MURALIS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
NaCl 30.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Methanol 5.0 ml  
Vitamin B<sub>12</sub> 20.0 µg  
Distilled water 1000.0 ml  
pH 8.0  
Autoclave at 121°C for 15 min. Methanol and vitamin B<sub>12</sub> added from stock solution (sterilized by filtration) after autoclaving.

#### **250. MODIFIED MEDIUM FOR MARINE METHYLOTROPHIC BACTERIA WITH YEAST EXTRACT AND BIOTIN**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Yeast extract 0.1 g  
Methanol 10.0 ml  
Biotin 0.01 mg  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C 15 min, methanol and biotin sterilize by filtration.

#### **251. CHELATIVORANS OLIGOTROPHICUS MEDIUM**

Na<sub>4</sub>EDTA 1.0 g  
Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 4.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.26 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.4 g  
Trace element solution (see below) 2.0 ml  
Vitamin solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
pH 7.0  
*Trace element solution:*  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
H<sub>3</sub>BO<sub>3</sub> 60.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 120.0 mg  
ZnCl<sub>2</sub> 70.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 25.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 15.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 25.0 mg  
HCl, 0.05 molar 1000.0 ml

*Vitamin solution:*

Pyridoxine-HCl 100.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 50.0 mg  
Riboflavin 50.0 mg  
Nicotinic acid 50.0 mg  
Pantothenic acid 50.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Lipoic acid 50.0 mg  
Nicotine amide 50.0 mg  
Vitamin B<sub>12</sub> 50.0 µg  
Biotin 20.0 mg  
Folic acid 20.0 mg  
Distilled water 1000.0 ml

Autoclave at 121°C 15 min, vitamin solution stir for some hours and sterilize by filtration.

**252. MODIFIED METHYLOTROPH MEDIUM 1 WITH KCNS**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
KCNS 0.24 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Methanol 5.0 ml or  
Methylamine 3.0 g  
Distilled water 1000.0 ml  
pH 7.0

Autoclave at 121°C 15 min, methanol and methylamine sterilize by filtration.

**253. MODIFIED MEDIUM FOR METHANOTROPHIC BACTERIA WITH ETHANOL**

KNO<sub>3</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.7 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>HPO<sub>4</sub> × 2 H<sub>2</sub>O 1.5 g  
Trylon B 5.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 mg  
Ethanol 5.0 ml  
Distilled water 1000.0 ml  
pH 6.7 - 7.1

Autoclave at 121°C 15 min, ethanol sterilize by filtration.

Cultivation under mixture of methane and air (1:1).

**254. MODIFICATION OF TWEEN-80 MEDIUM FOR MILK-ACID BACTERIA (10% NaCl)**

Beef extract 1.2 g

Yeast extract 5.0 g  
Glucose 2.5 g  
Tween-80 1.0 ml  
K<sub>2</sub>HPO<sub>4</sub> 2.0 g  
Na-acetate × 3 H<sub>2</sub>O 5.0 g  
NaCl 60.0 g  
NH<sub>4</sub>-citrate 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
MnSO<sub>4</sub> × 4 H<sub>2</sub>O 0.05 g  
Agar 5.0 g  
Distilled water 1000.0 ml  
pH 6.0-6.5  
Autoclave at 111°C for 30 min.

### **255. MINERAL MEDIUM FOR *METHYLOMICROBIUM KENYENSE***

Na<sub>2</sub>CO<sub>3</sub> 2.3 g  
NaHCO<sub>3</sub> 7.0 g  
K<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 0.5 g  
KNO<sub>3</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g  
NaCl 5.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml

#### *Trace elements solution:*

EDTA 5.0 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

Prepare the medium without NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>. Basal medium, trace element solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> autoclave at 121°C 15 min. When preparing liquid media cool the mineral salts solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> to room temperature before mixing. For solid medium add 2.0 % agar to the mineral salt solution and autoclave. Cool the NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> stock solution and agar to 50-55°C before mixing.

The pH of the complete medium should be at 10.0.

### **256. MODIFIED METHYLOTROPH MEDIUM 1 WITH YEAST AUTOLYZATE**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Methanol 5.0 ml or Methylamine 3.0 g  
Yeast autolizate 1.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C 15 min, methanol and methylamine sterilize by filtration.

### **257. MODIFIED STARCH AMMONIA AGAR (10% NaCl)**

Starch (soluble) 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0g  
NaCl 100.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
CaCO<sub>3</sub> 2.0 g  
Salt solution (see below) 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
*Salt solution:*  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Distilled water 100.0 ml  
pH 7.0-7.4  
Autoclave separately at 121°C 15 min.

### **258. NOCARDIOIDES AQUATICUS MEDIUM**

Base medium (see below) 600.0 ml  
Artificial sea water (see below) 400.0 ml  
pH 7.5  
*Base medium:*  
Peptone Bacto 0.25 g  
Yeast extract Bacto 0.25 g  
Glucose solution (2.5%) 10.0 ml  
Staley's vitamin solution, double concentration (see below) 5.0 ml  
Hutner's basal salts medium (see below) 20.0 ml  
Agar Bacto 15.0 g  
Distilled water 965.0 ml  
*Artificial sea water (AWS):*  
NaCl 23.477 g  
Na<sub>2</sub>SO<sub>4</sub> 3.917 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.981 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.1 g  
NaHCO<sub>3</sub> 192.0 mg  
KCl 664.0 mg  
KBr 6.0 mg  
H<sub>3</sub>BO<sub>3</sub> 26.0 mg  
SrCl<sub>2</sub> 24.0 g  
NaF 3.0 mg  
Distilled water 1000.0 ml  
*Hutner's basal salts medium:*  
Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 99.0 mg  
Metal salt solution "44" (see below) 50.0 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Adjust volume to 1000.0 ml with distilled water.  
*Metal solution "44":*  
Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg



CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

*Staley's vitamin solution, double concentration:*

Biotin 4.0 mg  
Folic acid 4.0 mg  
Pyridoxine-HCl 20.0 mg  
Riboflavin 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 10.0 mg  
Nicotinamide 10.0 mg  
D-Ca-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 0.2 mg  
*p*-Aminobenzoic acid 10.0 mg  
Distilled water 1000.0 ml

Autoclave Base medium (except glucose solution and vitamin solution) and Artificial sea water separately at 121°C 15 min. After cooling to 60°C aseptically add to the Base medium glucose solution (sterilize by filtration) and vitamin solution (sterilize by filtration). Store vitamin solution in the dark and cold (5°C). Add 600.0 ml Base medium to 400 ml Artificial sea water and mix thoroughly.

#### **259. ISP-2 MEDIUM WITH 5% NaCl**

Yeast extract 4.0 g  
Malt extract 10.0 g  
Glucose (D-glucose) 4.0 g  
Agar 15.0-20.0 g  
NaCl 50.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Autoclave at 111°C for 30 min.

#### **260. V-8 MEDIUM**

Commercial V8 vegetable juice 175.0 ml  
CaCO<sub>3</sub> 3.0 g  
Agar 20.0 g  
Distilled water to 1000.0 ml  
pH 6.4  
Autoclave at 111°C for 30 min.

#### **261. ROSEINATRONOBACTER MONICUS MEDIUM**

NH<sub>4</sub>Cl 0.4 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 0.5 g  
NaNO<sub>3</sub> 0.4 g  
NaCl 40.0 g  
KCl 0.5 g  
NaHCO<sub>3</sub> 10.0 g  
Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 1.0 g  
Vitamin B<sub>12</sub> 10.0 µg  
Sodium pyruvate 1.0 g

Sodium acetate 1.0 g  
Peptone 1.0 g  
Yeast extract 1.0 g  
Trace element solution *SL-10* 1.0 ml

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>2</sub> × 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Autoclave the medium, trace element solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> separately at 121°C for 15 min and add to the medium. Add the vitamin B<sub>12</sub> solution from a filter-sterilized stock solution to the autoclaved, cooled medium.

Final pH 9.0 - 9.5

## **262. MINERAL MEDIUM**

KNO<sub>3</sub> 250.0 mg

KH<sub>2</sub>PO<sub>4</sub> 100.0 mg

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 50.0 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg

Trace elements solution 1.0 ml

Distilled water 1000.0 ml

*Trace elements solution:*

EDTA 5.0 g

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g

Distilled water 1000.0 ml

Autoclave at 121°C for 15 min.

Final pH 5.5-6.0

## **263. SINGULISPHAERA MEDIUM**

N-acetylglucosamine 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.1 g

*Hutner's basal salts medium* 20.0 ml

Peptone 0.1 g

Yeast extract 0.1 g

Agar-agar (Difco) 18.0 g

Distilled water 1000.0 ml

Adjust pH to 5.8

*Hutner's basal salts medium:*

Nitrilotriacetic acid (NTA) 10.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 99.0 mg  
Metal salt solution “44” (see below) 50.0 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Adjust volume to 1000.0 ml with distilled water.

*Metal solution “44”:*

Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.  
Autoclave at 121°C 15 min.

#### **264. ROSEOCOCCUS SUDUNTUYENSIS MEDIUM**

NH<sub>4</sub>Cl 0.4 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 0.5 g  
NaCl 2.0 g  
KCl 0.5 g  
NaHCO<sub>3</sub> 5.0 g  
Na<sub>2</sub>CO<sub>3</sub> 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 1.0 g  
Vitamin B<sub>12</sub> 10.0 µg  
Na-pyruvate 1.0 g  
Na-acetate × 3 H<sub>2</sub>O 1.0 g  
Peptone 1.0 g  
Yeast extract 1.0 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Final pH 8.5  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>2</sub> × 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Autoclave the medium, trace element solution,  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  separately at  $121^\circ\text{C}$  for 15 min and add to the medium. Add the vitamin  $\text{B}_{12}$  solution from a filter sterilized stock solution to the cooled medium.

### **265. ECTOTHIORHODOSPIRA VARIABILIS MEDIUM**

$\text{NaCl}$  50.0 g  
 $\text{Na}_2\text{CO}_3$  5.0 g  
 $\text{NaHCO}_3$  15.0 g  
 $\text{KCl}$  0.1 g  
 $\text{K}_2\text{HPO}_4$  0.5 g  
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.2 g  
 $\text{NH}_4\text{Cl}$  0.5 g  
 $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.5 g  
 $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{H}_2\text{O}$  0.5 g  
Yeast extract 0.1 g  
Na-acetate  $\times 3 \text{H}_2\text{O}$  0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml

Vitamin  $\text{B}_{12}$  20.0  $\mu\text{g}$   
Distilled water 1000.0 ml

*Trace element solution SL-10:*

$\text{HCl}$  (25%; 7.7 M) 10.0 ml  
 $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.5 g  
 $\text{ZnCl}_2$  70.0 mg  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg  
 $\text{H}_3\text{BO}_3$  6.0 mg  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  36.0 mg  
Distilled water 990.0 ml

*The trace element solution preparation:*  $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  is dissolved firstly in  $\text{HCl}$ , and then is mixed with water and other salts are dissolved in the sequence indicated.

Prepare and boil the medium in the absence of  $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ ,  $\text{NaHCO}_3$ , vitamin  $\text{B}_{12}$ ,  $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ . Cool under a stream of nitrogen and add the remaining components, except vitamin  $\text{B}_{12}$  and  $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{H}_2\text{O}$ . Dispense into tubes or bottle fitted with rubber stoppers and autoclave. Add the vitamin  $\text{B}_{12}$  (filter sterilized) and  $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{H}_2\text{O}$  from sterile stock solutions.

Autoclave at  $121^\circ\text{C}$  for 15 min.

Final pH 9.0 – 9.5

### **266. ACIDISOMA MEDIUM**

$\text{KH}_2\text{PO}_4$  0.1 g  
 $(\text{NH}_4)_2\text{SO}_4$  0.25 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.05 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.02 g  
Trace elements solution (see below) 1.0 ml

Yeast extract 0.1 g  
Na gluconate 0.5 g  
Distilled water 1000.0 ml

*Trace element solution s:*

EDTA 5.0 g  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  2.0 g  
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.02 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g

Distilled water 1000.0 ml

Autoclave the base medium and trace element solution separately at 121°C for 15 min. Adjust to pH 5.0 – 5.5.

### **267. GRANULICELLA MEDIUM**

Glucose 0.5 g

Yeast extract 0.1 g

Casamino acids 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.04 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

The medium may be solidified with 15 g/l agar.

Final pH 4.5 – 5.2

Autoclave at 121°C for 15 min.

### **268. ZAVARZINELLA FORMOSA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.05 g

NaCl 0.01 g

N-acetylglucosamine 1.0 g

Glucose 0.5 g

Peptone 0.1 g

Yeast extract 0.1 g

Casamino acids 0.1 g

“Metals 44” 1.0 ml

Agar (for solid medium) 15.0 g

Distilled water 1000.0 ml

Final pH 5.8 – 6.0

*Metal solution “44”:*

Na-EDTA 250.0 mg

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg

MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg

Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg

Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

Autoclave the medium and trace element solution separately at 121°C for 15 min.

### **269. THIOTHRUX MEDIUM**

NH<sub>4</sub>Cl 0.2 g

K<sub>2</sub>HPO<sub>4</sub> 10.0 mg

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 mg

CaSO<sub>4</sub> (saturated solution) 20.0 ml

Trace element solution (see below) 5.0 ml

Na-acetate × 3 H<sub>2</sub>O 0.1 g

Agar (if necessary) 12.0 g

Na<sub>2</sub>S × 9 H<sub>2</sub>O 10% (w/v) solution 3.0 ml

Distilled water 1000.0 ml

Adjust pH to 7.5 before autoclaving.

*Trace element solution:*

EDTA 0.2 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.7 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.01 g

MnSO<sub>4</sub> × 4 H<sub>2</sub>O 0.02 g

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 5.0 µg

H<sub>3</sub>BO<sub>3</sub> 10.0 mg

Co(NO<sub>3</sub>)<sub>2</sub> 1.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 1.0 mg

Distilled water 1000.0 ml

Basal medium and Na<sub>2</sub>S × 9 H<sub>2</sub>O solution autoclave separately at 121°C 15 min.

### **270. TRYPTICASE SOY BROTH AGAR**

Trypticase Soy Broth (BBL 11768, Oxoid CM129 or Merck 5459) 30.0 g or

Pancreatic digest of casein 18.0 g

Papaic digest of soybean 6.0 g

NaCl 6.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.3

Autoclave at 121°C for 15 min.

### **271. MARINE SPIROCHETE MEDIUM**

Tryptone 2.0 g

Yeast extract 1.0 g

Na-Thioglycolate 1.0 g

Resazurin 0.5 mg

Charcoal-filtered, natural seawater 800.0 ml

Distilled water 200.0 ml

Dissolve ingredients (except thioglycolate), boil medium for 3 min, then cool to room temperature under N<sub>2</sub> gas atmosphere. Add thioglycolate and adjust pH of medium to 7.5 with 10 N KOH.

Dispense under N<sub>2</sub> gas atmosphere in culture vessels and autoclave at 121°C for 15 min. Prepare 10% cellobiose solution (10.0 g in 100.0 ml distilled water) under nitrogen atmosphere, filter-sterilize and add 0.2 ml to 10.0 ml autoclaved medium.

### **272. ECTOTHIORHODOSPIRA MAGNA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g

NaCl 30.0 g

NH<sub>4</sub>Cl 0.5 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

Na-acetate × 3 H<sub>2</sub>O 1.0 g

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 0.5 g

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

NaHCO<sub>3</sub> 5.0 g

Na<sub>2</sub>CO<sub>3</sub> 5.0 g

Yeast extract 0.1 g

Vitamin B<sub>12</sub> 10.0 µg

Trace elements solution (see below) 1.0 ml

Distilled water 1000.0 ml

pH 9.0-9.5

*Trace elements solution:*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.2 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.03 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml  
pH 3.0-4.0

Prepare the medium without the NaHCO<sub>3</sub>, Na<sub>2</sub>S × 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> and vitamin B<sub>12</sub>, under a nitrogen atmosphere.

Autoclave at 121°C for 15 min and add the vitamin B<sub>12</sub> from a filter-sterilized stock solution and the NaHCO<sub>3</sub> (autoclaved in sealed, half full vessels), Na<sub>2</sub>S × 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> from autoclaved at 121°C for 15 min stock solutions. The final pH of the medium should be 9.0-9.5.

### **273. LAMPROBACTER MODESTOHALOPHILUS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NaCl 40.0 g  
NH<sub>4</sub>Cl 0.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na-acetate × 3 H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
NaHCO<sub>3</sub> 1.5 g  
Yeast extract 0.1 g  
Vitamin B<sub>12</sub> 20.0 µg  
Trace elements solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
pH 7.5

*Trace elements solution:*

EDTA 5.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.2 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.03 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml  
pH 3.0-4.0

Prepare the medium without the NaHCO<sub>3</sub>, Na<sub>2</sub>S × 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> and vitamin B<sub>12</sub>, under a nitrogen atmosphere. Autoclave at 121°C for 15 min and add the vitamin B<sub>12</sub> from a filter-sterilized stock solution and the NaHCO<sub>3</sub> (autoclaved in sealed, half full vessels), Na<sub>2</sub>S × 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> from autoclaved at 121°C for 15 min stock solutions. The final pH of the medium should be 9.0-9.5.

### **274. GRANULICELLA PALUDICOLA MEDIUM**

Fructose 0.5 g  
Yeast extract 0.05 g  
Casamino acids 0.05 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.04 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g

Distilled water 1000.0 ml

The medium may be solidified with 15 g/l agar. Adjust to pH 4.0 – 5.0 with alginic acid.

Autoclave at 121°C for 15 min.

### **275. TELMATOCOLA MEDIUM**

Mineral salts solution:

$\text{KH}_2\text{PO}_4$  0.1 g

$(\text{NH}_4)_2\text{SO}_4$  0.1 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.02 g

*Hutner's* basal salts medium 1.0 ml

*Staley's* vitamin solution 1.0 ml

Yeast extract 0.1 g

Glucose (1% v/v) 10.0 ml

Distilled water 1000.0 ml

Trace elements solution 44:

*Hutner's basal salts medium*:

Nitrilotriacetic acid (NTA) 10.0 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  29.7 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  3.335 g

$(\text{NH}_4)_6\text{MoO}_7\text{O}_{24} \times 4 \text{H}_2\text{O}$  9.25 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  99.0 mg

"Metals 44" 50.0 ml

Distilled water 950.0 ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8.

"Metals 44":

Na-EDTA 250.0 mg

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  1095.0 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  500.0 mg

$\text{MnSO}_4 \times \text{H}_2\text{O}$  154.0 mg

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$  39.2 mg

$\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$  24.8 mg

$\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$  17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated  $\text{H}_2\text{SO}_4$  to retard precipitation of the heavy metal ions.

*Staley's* vitamin solution:

Vitamin  $\text{B}_{12}$  0.1 mg

Biotin 2.0 mg

Thiamine-HCl  $\times 2 \text{H}_2\text{O}$  5.0 mg

Ca-pantothenate 5.0 mg

Folic acid 2.0 mg

Riboflavin 5.0 mg

Nicotinamide 5.0 mg

*p*-Aminobenzoic acid 5.0 mg

Pyridoxine hydrochloride 10.0 mg

Distilled water 1000.0 ml

Prepare the liquid medium. Adjust to pH 4.8 - 5.5. Dispense the medium into serum bottles under gassing with  $\text{CO}_2$  (5% v/v). Autoclave at 121°C for 15 min. Before inoculation add from filter-sterilized *Staley's* vitamin solution and autoclaving at 121°C for 15 min glucose.

### **276. PROTEINIVORAX MEDIUM**

$\text{KH}_2\text{PO}_4$  0.3 g



MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.12 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 30.0 mg  
NaCl 20.0 g  
Tryptone 3.0 g  
Trace element solution *SL-10* 1.0 ml

Na<sub>2</sub>CO<sub>3</sub> 60.0 g  
NaHCO<sub>3</sub> 50.0 g  
Na-Thioglycolate 1.0 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>2</sub> × 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Dissolve ingredients (except carbonates and thioglycolate), boil medium for 1 min, then cool to room temperature under N<sub>2</sub> gas atmosphere. Add carbonates and adjust pH to 8.5 – 9.0. Dispense under same gas atmosphere in culture vessels and autoclave. After autoclaving at 121°C for 15 min add thioglycolate from a sterile anoxic stock solution sterilized by filtration.

### **277. MS1 MEDIUM**

NH<sub>4</sub>Cl 2.0 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

K<sub>2</sub>SO<sub>4</sub> 0.5 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.5 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 2.0 mg

H<sub>3</sub>BO<sub>3</sub> 0.06 mg

ZnCl<sub>2</sub> 20.0 mg

MnSO<sub>4</sub> × H<sub>2</sub>O 1.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.05 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.3 mg

Na-glutamate 10.0 g

Distilled water 1000.0 ml

pH 7.0-7.5

Autoclave at 121°C for 15 min.

### **278. MODIFIED S MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.14 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

NH<sub>4</sub>Cl 0.25 g

KCl 0.36 g

NaCl 6.0

Yeast extract 0.1 g

Trace elements solution (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution:*

$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$  (Mohr's salt) 784.0 mg

HCl (concentrated) 5.0 ml

$\text{CoCl}_2 \times \text{H}_2\text{O}$  238.0 mg

$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$  395.0 mg

$\text{Na}_2\text{MoO}_4 \times \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$  33.0 mg

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  144.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{Na}_2\text{SeO}_4$  94.0 mg

$\text{HBO}_3$  6.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  99.0 mg

Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with water and other salts are dissolved in the sequence indicated.

*Vitamin solution:*

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine 100.0 mg

Riboflavin 50.0 mg

Pantotenoic acid 50.0 mg

*p*-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B<sub>12</sub> 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution and trace elements from sterile stock solution.

### **279. M3 MEDIUM**

$\text{KH}_2\text{PO}_4$  0.1 g

$\text{NH}_4\text{Cl}$  0.2 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.02 g

Yeast extract 0.1 g

Glucose (or malate) 0.5 g

Trace element solution 'SLA' (see below) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution SLA:*

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.8 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  250.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  10.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  10.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  70.0 mg

$\text{ZnCl}_2$  100.0 mg

$\text{H}_3\text{BO}_3$  500.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  30.0 mg

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  10.0 mg

Distilled water 1000.0 ml

Autoclave basal medium and trace element solution separately at 121°C 15 min.

pH 5.8-6.0

## 280. *PLANCTOMYCES* MEDIUM

KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
NaCl 0.01 g  
N-acetylglucosamine 1.0 g  
Glucose 0.5 g  
Yeast extract 0.2 g  
*Hutner's* basal salts (see below) 1.0 ml  
*Staley's* vitamin solution (see below) 1.0 ml  
Phytigel (for solid medium) 8.0 g  
Distilled water 1000.0 ml  
Adjust pH to 5.8-6.5

### *Hutner's basal salts*

Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 99.0 mg  
Metal salt solution "44" (see below) 50.0 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Adjust volume to 1000.0 ml with distilled water.

### *Metal solution "44":*

Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

### *Staley's vitamin solution:*

Vitamin B<sub>12</sub> 0.1 mg  
Biotin 2.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Ca-pantothenate 5.0 mg  
Folic acid 2.0 mg  
Riboflavin 5.0 mg  
Nicotinamide 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Pyridoxine-HCl 10.0 mg  
Distilled water 1000.0 ml

Store in the dark and cold (5°C).

Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution.

## 281. *AZOSPIRILLUM AMAZONENSE* MEDIUM

K<sub>2</sub>HPO<sub>4</sub> 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.6 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.02 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 0.01 g  
Bromothymol blue (0.5% in 0.2N KOH) 5.0 ml  
Sucrose 5.0 g  
Distilled water 1000.0 ml  
Adjust pH to 6.0  
For semisolid medium, add 0.5 g of agar; for solid medium, add 15 g of agar.  
Autoclave at 111°C for 30 min.

### **282. AZOSPIRILLUM HALOPRAEFERENS MEDIUM**

Beef extract 1.0 g  
Yeast extract 2.0 g  
Peptone 5.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.4  
Autoclave at 121°C 15 min.

### **283. AZOSPIRILLUM VM MEDIUM**

Döbereiner's basic 10.0 ml  
Fe<sub>3</sub> EDTA 0.66% (w/v) 10.0 ml  
NH<sub>4</sub>Cl 0.5 g  
NaCl 1.0 g  
Yeast extract 1.0 g  
Peptone 3.0 g  
Phosphate buffer pH 6.8 3.0 ml  
Agar 15.0 g  
Distilled water 1000.0 ml  
*Döbereiner's basic:*  
MgSO<sub>4</sub> 20.0 g  
NaCl 10.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 2.64 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.2 g  
MnSO<sub>4</sub> × 2 H<sub>2</sub>O 1.0 g  
Distilled water 1000.0 ml  
*Phosphate buffer pH 6.8:*  
KH<sub>2</sub>PO<sub>4</sub> 0.6 g  
K<sub>2</sub>HPO<sub>4</sub> 0.4 g  
Distilled water 3.0 ml  
Autoclave basal medium, Fe<sub>3</sub>EDTA solution, Döbereiner's basic and phosphate buffer separately at 121°C 15 min.

### **284. METHYLOCYSTIS MEDIUM**

KNO<sub>3</sub> 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.05 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
NaCl 0.01 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
Final pH 5.8-6.2.  
*Trace element solution:*  
EDTA 5.0 g

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  2.0 g

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.02 g

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  0.2 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  0.03 g

Distilled water 1000.0 ml

Dispense the medium into serum bottles with a medium to headspace ratio of about 1:4. Autoclave at 121°C for 15 min. Add 20% methane to the gas phase. Incubate the cultures with shaking.

### **285. TELMATOBACTER BRADUS MEDIUM**

Yeast extract 0.05 g

Proteose peptone 0.05 g

Casamino acids 0.05 g

Glucose 0.05 g

Soluble starch 0.05 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.05 g

Distilled water 1000.0 ml

Adjust pH to 4.5 - 5.0 with alginic acid (or MES), fill tubes for anaerobes (Hungate) half with medium, close tightly. Autoclave at 121°C for 15 min. Do not shake.

### **286. LARKINELLA ARBORICOLA MEDIUM**

$\text{KH}_2\text{PO}_4$  0.1 g

N-acetylglucosamine 1.0 g

Peptone 0.1 g

Yeast extract 0.1 g

Casamino acids 0.1 g

Glucose 0.5 g

*Hutner's salts* (see below) 20.0 ml

Distilled water 980.0 ml

*Hutner's salts:*

Nitrilotriacetic acid (NTA) 10.0 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  29.7 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  3.335 g

$(\text{NH}_4)_6\text{MoO}_7\text{O}_{24} \times 4 \text{H}_2\text{O}$  9.25 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  99.0 mg

"Metals 44" 50.0 ml

Distilled water 950.0 ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or  $\text{H}_2\text{SO}_4$ .

*"Metals 44":*

Na-EDTA 250.0 mg

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  1095.0 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  500.0 mg

$\text{MnSO}_4 \times \text{H}_2\text{O}$  154.0 mg

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$  39.2 mg

$\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$  24.8 mg

$\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$  17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated  $\text{H}_2\text{SO}_4$  to retard precipitation of the heavy metal ions.

The medium may be solidified by adding 15 g/l agar. Final pH should be 6.0 – 6.5. Autoclave at 121°C for 15 min.

**287. METHYLOCAPSA AUREA MEDIUM**

NaNO<sub>3</sub> 200.0 mg  
MgSO<sub>4</sub> × 6 H<sub>2</sub>O 200.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 40.0 mg  
Fe-EDTA solution (see below) 3.0 ml  
Phosphate buffer pH 5.8 100 mM (see below) 10.0 ml  
Trace elements solution (see below) 1.0 ml  
Distilled water 986.0 ml

*Trace elements solution:*

EDTA 5.0 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

*Fe-EDTA solution:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.54 g  
Na<sub>2</sub>EDTA 2.06 g  
Distilled water 1000.0 ml

*Phosphate buffer 100 mM:*

K<sub>2</sub>HPO<sub>4</sub> × 3 H<sub>2</sub>O 1.71 g  
KH<sub>2</sub>PO<sub>4</sub> 12.58 g  
Distilled water 1000.0 ml

Autoclave at 121°C for 15 min. Final pH 5.0-5.8. 20-30% methane is added to the gas phase. The strain should be grown with shaking.

**288. ACIDICAPSA BOREALIS MEDIUM**

Yeast extract 0.05 g  
Proteose peptone 0.05 g  
Casamino acids 0.05 g  
Glucose 0.2 g  
Soluble starch 0.05 g  
Na-pyruvate 0.03 g  
K<sub>2</sub>HPO<sub>4</sub> 0.03 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.05 g  
Distilled water 1000.0 ml  
Adjust pH to 5.0 – 5.5. Autoclave at 121°C for 15 min.

**289. MEDIUM FOR ECTOTHIORHODOSPIRA**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
NH<sub>4</sub>Cl 0.8 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
NaCl 30.0 g  
Na<sub>2</sub>SO<sub>4</sub> 20.0 g  
Na<sub>2</sub>CO<sub>3</sub> 6.0 g  
Trace element solution (see below) 1.0 ml  
Yeast extract 0.5 g  
Na-succinate 1.0 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g  
Distilled water 800.0 ml  
Adjust pH to 8.5.

*Trace element solution:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.8 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 250.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 10.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 70.0 mg  
ZnCl<sub>2</sub> 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 500.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 30.0 mg  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 10.0 mg  
Distilled water 1000.0 ml

For dissolving adjust pH to about 3 with 1 N HCl.

*Vitamin solution:*

Biotin 10.0 mg  
Nicotinamide 35.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 30.0 mg  
*p*-Aminobenzoic acid 20.0 mg  
Pyridoxal-HCl 10.0 mg  
Ca-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 5.0 mg  
Distilled water 100.0 ml

*Bicarbonate solution:*

NaHCO<sub>3</sub> 14.0 g  
H<sub>2</sub>O 200.0 ml

Autoclave at 121°C for 15 minutes in screw-capped bottles. After sterilization add 1 ml/l filter-sterilized vitamin solution and 200 ml of filter-sterilized bicarbonate solution.

**290. MARINOSPIRILLUM CELERE MEDIUM**

NH<sub>4</sub>Cl 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NaCl 25.0 g  
Na<sub>2</sub>SO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
KCl 0.5 g  
Yeast extract 1.0 g  
Peptone 1.0 g  
Lactate 1.0 g  
Vitamin B<sub>12</sub> 15.0 µg  
Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
NaHCO<sub>3</sub> 5.0 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

Distilled water 1000.0 ml  
HCl (25%) 7.7 ml  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Autoclave the Na<sub>2</sub>CO<sub>3</sub>, NaHCO<sub>3</sub>, lactate and the medium separately at 121°C for 15 min and combine

after cooling. Vitamin B<sub>12</sub> is added from a sterilized by filtration stock solution. The final pH of the medium should be 9.5. Add 15.0 g/l agar for solid media.

### **291. PROSTHECOMICROBIUM MEDIUM**

NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 0.3 g

Na<sub>2</sub>HPO<sub>4</sub> 0.71 g

KH<sub>2</sub>PO<sub>4</sub> 0.36 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.3 g

Trace element solution (see below) 1.0 ml

Yeast extract 0.3 g

Glucose 0.3 g

Distilled water 1000.0 ml

*Trace element solution:*

Na-EDTA 0.25 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1.1 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

MnSO<sub>4</sub> × H<sub>2</sub>O 0.154 g

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.04 g

Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 0.025 g

Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 0.018 g

Distilled water 100.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

Adjust pH to 7.0. Autoclave at 121°C for 15 min.

### **292. R2A AGAR MEDIUM**

Bacto yeast extract 0.5 g

Proteose peptone 0.5 g

Casamino acids 0.5 g

Glucose 0.5 g

Soluble starch 0.5 g

Na-pyruvate 0.3 g

KH<sub>2</sub>PO<sub>4</sub> 0.3 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 50 mg

Agar 15.0 g

Distilled water 1000.0 ml. Autoclave at 121°C for 15 min.

### **293. TPT 18 MEDIUM**

Glucose 0.5 g

Yeast extract 0.1 g

Casitone (pancreatic digest of casein) 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 50 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 20 mg

Agar (for solid medium) 14.0 g

Distilled water 1000.0 ml

Adjust to pH 6.0. Autoclave at 121°C for 15 min.

### **294. METHYLOMICROBIUM JAPANENSE MEDIUM**

NH<sub>4</sub>NO<sub>3</sub> 0.1 g

KH<sub>2</sub>PO<sub>4</sub> 0.01 g

Fe(III)-EDTA 2.5 mg

Vitamin solution (see below) 10.0 ml

Trace elements solution (see below) 10.0 ml

Methanol 2.0 ml



Seawater 980.0 ml

pH 8.1

*Vitamin solution*

Vitamin B<sub>12</sub> 0.275 mg

Biotin 0.25 mg

Thiamine-HCl 50 mg

Distilled water 1000.0 ml

*Trace elements solution*

Na-EDTA 37.2 mg

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.025 mg

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.575 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.455 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.06 mg

(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> × 4 H<sub>2</sub>O 0.027 mg

Distilled water 1000.0 ml

Autoclave at 121°C for 15 min, add methanol and vitamin solution sterilized by filtration.

### **295. NAUTILIA MEDIUM**

Synthetic seawater (2 × conc.) (see below) 500.0 ml

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

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

Resazurin 0.5mg

Distilled water 400.0 ml

*Synthetic seawater (2 × conc.):*

NaCl 55.4 g

SrCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 14.0 g

KI 0.1 mg

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 11.0 g

Na<sub>3</sub>-citrate 20.0 mg

KCl 1.3 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.5 g

NaBr 0.2 g

H<sub>3</sub>BO<sub>3</sub> 0.06 g

Distilled water 1000.0 ml

Sulfur, powdered 10.0 g

Na-formate (20% w/v) 15.0 ml

NaHCO<sub>3</sub> (5% w/v) 50.0 ml

Trace element solution (see below) 10.0 ml

Selenite-tungstate solution (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O (3% w/v) 20.0

*Trace element solution:*

Nitrilotriacetic acid 1.5 g

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

MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g

NaCl 1.0 g

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

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

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

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

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

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

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

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  0.01 g

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.03 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  0.3 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$  0.4 mg

Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl  $\times 2 \text{H}_2\text{O}$  5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  3.0 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$  4.0 mg

Distilled water 1000.0 ml

Prepare medium anoxically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. Vitamin solution are prepared under N<sub>2</sub> gas atmosphere and sterilized by filtration. Na-formate, NaHCO<sub>3</sub>, trace element solution, selenite-tungstate solution and Na<sub>2</sub>S  $\times 9 \text{H}_2\text{O}$  solution sterilize separately under 100% N<sub>2</sub> gas at 121°C for 15 min. Place the sulfur in screw-capped tubes or bottles and autoclave at 112°C for 15 min. Before use, aseptically layer the sulfur onto the surface of autoclaved liquid basal medium.

### **296. ACIDICAPSA LIGNI MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g

MgSO<sub>4</sub>  $\times 7 \text{H}_2\text{O}$  0.1 g

NaNO<sub>3</sub> 0.1 g

CaCl<sub>2</sub>  $\times 2 \text{H}_2\text{O}$  0.1 g

Yeast extract 0.1 g

Cellobiose 0.1 g

Distilled water 1000.0 ml

Adjust pH to 5.0 – 5.5. Autoclave at 121°C for 15 min.

### **297. EDAPHOBACTER LICHENICOLA MEDIUM**

MgSO<sub>4</sub>  $\times 7 \text{H}_2\text{O}$  0.8 g

KNO<sub>3</sub> 0.2 g

CaCl<sub>2</sub>  $\times 2 \text{H}_2\text{O}$  0.02 g

Glucose 0.8 g

Yeast extract 0.1 g

Casamino acids 0.1 g

Phytigel 9.0g

Distilled water 1000.0 ml

Adjust to pH 5.0-5.6. Autoclave at 121°C for 15 min.

### **298. METHYLOROSULA POLARIS MEDIUM**

*Mineral salts solution:*

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.05 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.04 g  
KH<sub>2</sub>PO<sub>4</sub> 0.07 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Trace element solution:*

Na<sub>2</sub>EDTA 500.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 200.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 10.0 mg  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 3.0 mg  
H<sub>3</sub>BO<sub>3</sub> 30.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 3.0 mg  
Distilled water 1000.0 ml

Prepare the liquid medium. Adjust to pH 6.0. Dispense the medium in tubes or flasks fitted with screw caps. Autoclave at 121°C for 15 min. Before inoculation add filter sterilized CH<sub>3</sub>OH to a final concentration of 0.5% v/v.

**299. SULFITOBACTER PONTIACUS MEDIUM**

Na-acetate 20.0 mM  
HEPES 8.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
NH<sub>4</sub>Cl 0.5 g  
Yeast extract 1.0 g  
Peptone 0.5 g  
NaCl 15.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
Trace element solution (see below) 1.0 ml  
Biotin 0.1 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Prepare the HEPES, NaCl, K<sub>2</sub>HPO<sub>4</sub> and NH<sub>4</sub>Cl as a basal salt solution, adjusting the pH to 7.5-7.8 with NaOH before sterilization. All other components are added from sterile stock solutions. Biotin solution is filter sterilized and all other solutions autoclave at 121°C for 15 min.

### **300. TELMATOSPIRILLUM MEDIUM**

K<sub>2</sub>HPO<sub>4</sub> 0.25 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> 0.1 g  
MgSO<sub>4</sub> 0.4 g  
Na-EDTA 0.01 g  
FeCl<sub>3</sub> × 6 H<sub>2</sub>O 1.0 mg  
KI 0.2 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.8 mg  
ZnSO<sub>4</sub> 0.8 mg  
H<sub>3</sub>BO<sub>3</sub> 0.1 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 mg  
Na-citrate 2.0 g  
Distilled water 1000.0 ml

Prepare the medium anaerobically under nitrogen. Adjust to pH 5.5 – 6.5 with 1M H<sub>3</sub>PO<sub>4</sub>. Dispense into serum bottles or Hungate tubes (under a nitrogen atmosphere) that can be sealed with rubber stoppers. Autoclave at 121°C for 15 min.

### **301. METHYLOFERULA STELLATA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 100.0 mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 100.0 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 50.0 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg  
NaCl 20.0 mg  
Fe-EDTA solution (see below) 3.0 ml  
Trace elements solution (see below) 1.0 ml  
Methanol 10.0 ml  
Distilled water 1000.0 ml

#### *Trace elements solution:*

EDTA 5.0 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

#### *Fe-EDTA solution:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.54 g  
Na<sub>2</sub>EDTA 2.06 g  
Distilled water 1000.0 ml  
Final pH 5.0-5.8.

The medium is fairly weakly buffered so the pH should be checked before and after autoclaving at 121°C for 15 min. Methanol is filter sterilized. The pH should be adjusted with H<sub>3</sub>PO<sub>4</sub> (sterilized if added to sterile medium). The strain should be grown with shaking.

### **302. PALUDIBACULUM FERMENTANS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.05 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
Yeast extract 0.1 g  
Glucose 0.5 g  
Phytigel 8.0 g  
Distilled water 1000.0 ml  
pH 5.5-6.0  
Autoclave at 121°C for 15 min.

### **303. AMMONIFEX MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.34 g  
NH<sub>4</sub>Cl 0.34 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
KCl 0.34 g  
Trace element solution (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
NaHCO<sub>3</sub> 1.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.7 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 0.5 g  
NaCl 25.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
Na-formate 2.0 g  
pH 7.0

#### *Trace element solution:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O (*Mohr's salt*) 784.0 mg  
CoCl<sub>2</sub> × H<sub>2</sub>O 238.0 mg  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 144.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg

#### *Vitamin solution:*

Biotin 20.0 mg  
Folic acid 20.0 mg  
Pyridoxine 100.0 mg  
Riboflavin 50.0 mg  
Pantotenoic acid 50.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Thiamine-HCl 50.0 mg  
Nicotinic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
Lipoic acid 50.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except Na-formate, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, vitamins and sulfide. Adjust pH to 3.5 with H<sub>2</sub>SO<sub>4</sub> and sparge medium with 100% CO<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., 10 ml medium in 25 ml Balch-type tubes) and autoclave at 121°C for 15 min. Add Na-formate, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas.

### **304. HALONATRONUM SACCHAROPHILUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 0.5 g  
KCl 0.2 g  
NaCl 50.0 g  
Na<sub>2</sub>CO<sub>3</sub> 68.0 g  
NaHCO<sub>3</sub> 38.0 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.7 g  
Yeast extract 0.2 g  
Sucrose 5.0 g  
Trace elements solution (see below) 1.0 ml  
Vitamin solution (see below) 10.0 ml  
Resazurin 0.01 g  
Distilled water 1000.0 ml  
Final pH 9.5-10.0

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Prepare the medium without adding the vitamins, sucrose, yeast extract, NH<sub>4</sub>Cl, Na<sub>2</sub>CO<sub>3</sub>, and NaHCO<sub>3</sub> using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH<sub>4</sub>Cl, Na<sub>2</sub>CO<sub>3</sub>, and NaHCO<sub>3</sub> after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave at 121°C for 15 min, and to the cooled medium add the vitamins (filter sterilized under N<sub>2</sub>), sucrose, yeast extract, and from anaerobic, sterile stock solutions.

### **305. GEOGLOBUS MEDIUM**

NaCl 18.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.0 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
NaHCO<sub>3</sub> 2.5 g  
Na-acetate × 3 H<sub>2</sub>O 1.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Yeast extract 0.2 g  
Vitamin solution (see below) 1.0 ml  
Amorphous Fe(OH)<sub>3</sub> sludge (see below) 200.0 ml  
Distilled water 800.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, acetate, phosphate, yeast extract, vitamins and amorphous iron. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Thereafter, dispense suspension under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add acetate, phosphate, yeast extract and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. The pH of the complete medium should be at 6.5 – 6.8. Amorphous Fe(OH)<sub>3</sub>: Slowly titrate 320 ml of a FeCl<sub>3</sub> × 6 H<sub>2</sub>O stock solution (60.0 g/l) with 10% (w/v) NaOH to pH 8.0-8.5 under agitation (use magnetic stirrer). Total amount of added NaOH approx. 80 – 100 ml. The precipitated Fe(OH)<sub>3</sub> should be stored at room temperature overnight with surface covered with water. Thereafter, centrifuge at 2000 rpm for 5 min and discard the supernatant. Resuspend the pellet in medium as described above.

### 306. *FERVIDICOCCUS* MEDIUM

NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.44 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.7 g  
NaCl 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Yeast extract 0.5 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 0.8 g  
Trypticase peptone 2.0 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, trypticase, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add Trypticase, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.0 - 6.1, if necessary.

### 307. *FERVIDOBACTERIUM* MEDIUM

NH<sub>4</sub>Cl 0.9 g  
NaCl 0.9 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KH<sub>2</sub>PO<sub>4</sub> 0.75 g  
K<sub>2</sub>HPO<sub>4</sub> 1.5 g  
Trace element solution (see below) 9.0 ml  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O solution (0.1% w/v in 0.1 N H<sub>2</sub>SO<sub>4</sub>) 3.0 ml



Yeast extract 3.0 g  
Trypticase peptone 10.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Vitamin solution (see below) 5.0 ml  
D-Glucose 5.0 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid (NTA) 12.8 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.17 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnCl<sub>2</sub> 0.1 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
NaCl 1.0 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.0 ml.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except vitamins, glucose and sulfide), sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 7.2 - 7.4.

**308. THERMOSIPHO AFFECTUS MEDIUM**

Sea salts (SIGMA) 30.0 g  
Yeast extract (OXOID) 0.1 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Trypticase peptone 2.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
D-Glucose 2.0 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.25 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except glucose, vitamins and sulfide, then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add glucose, vitamins and sulfide from sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.5 – 7.0, if necessary.

**309. MOORELLA MEDIUM**

NH<sub>4</sub>Cl 1.0 g  
Na<sub>2</sub>SO<sub>4</sub> 2.0 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
Yeast extract 1.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 2.0 g  
Vitamin solution (see below) 10.0 ml  
Na-DL-lactate 2.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.1 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins, lactate and sulfide), sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding vitamins (sterilized by filtration), lactate and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

### **310. CLOSTRIDIUM THERMOCELLUM MEDIUM**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.3 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 2.6 g  
KH<sub>2</sub>PO<sub>4</sub> 1.43 g  
K<sub>2</sub>HPO<sub>4</sub> 5.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.13 g  
Na<sub>2</sub>-β-glycerol phosphate × 4 H<sub>2</sub>O 6.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O solution (0.1% w/v in 0.1 N H<sub>2</sub>SO<sub>4</sub>) 1.1 ml  
L-Glutathione reduced 0.25 g  
Yeast extract 4.5 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Cellobiose 5.0 g  
Distilled water 1000.0 ml

Dissolve ingredients except cellobiose, sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Then adjust pH to 7.0 - 7.2, distribute under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Cellobiose is added to the sterile medium from an anoxic 10% (w/v) stock solution prepared under 100% N<sub>2</sub> gas and sterilized by filtration.

### **311. CLOSTRIDIUM MEDIUM I**

K<sub>2</sub>HPO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
NH<sub>4</sub>Cl 2.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
Yeast extract 5.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
D-Glucose 5.0 g  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

Dissolve ingredients except glucose, cysteine and sulfide. Sparge medium with 100% N<sub>2</sub> gas for 30 –

45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

### **312. THERMOTOGA NEAPOLITANA MEDIUM**

Starch, soluble 5.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
Trace element solution (see below) 15.0 ml  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O solution (0.1% w/v) 2.0 ml  
Sea water (see below) 250.0 ml  
Yeast extract 2.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 750.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Artificial sea water:*

NaCl 27.7 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 7.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 5.5 g  
KCl 0.65 g  
NaBr 0.1 g  
H<sub>3</sub>BO<sub>3</sub> 30.0 mg  
SrCl<sub>2</sub> × 6 H<sub>2</sub>O 15.0 mg  
Citric acid 10.0 mg  
KI 0.05 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 2.25 g  
Distilled water 1000.0 ml

Dissolve ingredients (except sulfide and cysteine) and adjust pH to 6.5. Boil medium for 1 min, then cool to room temperature under 100% N<sub>2</sub> gas atmosphere. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of volume and autoclave. Add sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 6.5, if necessary.

### **313. PYROCOCCLUS MEDIUM**

NaCl 13.85 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 2.75 g  
KCl 0.33 g  
NaBr 0.05 g  
H<sub>3</sub>BO<sub>3</sub> 15.0 mg  
SrCl<sub>2</sub> × 6 H<sub>2</sub>O solution (0.1% w/v) 7.0 ml  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 10.0 mg  
Citric acid solution (0.1% w/v) 5.0 ml  
KI solution (0.01% w/v) 0.5 ml  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.75 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O (0.1% w/v) 2.0 ml  
Trace element solution (see below) 10.0 ml  
Peptone (BD Bacto) 5.0 g  
Yeast extract (OXOID) 1.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Sulfur, powdered 30.0 g  
Neutralized sulfide solution 16.7 ml  
Distilled water 1000.0 ml

*Neutralized sulfide solution:*

Na<sub>2</sub>S × 9 H<sub>2</sub>O 3.0 g  
Distilled water 100.0 ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 N H<sub>2</sub>SO<sub>4</sub> drop-wise with a syringe without opening the bottle. Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients except sulfur and sulfide, adjust the pH to 6.5, and sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 1 - 2 hours on each of 3 successive days. Add neutralized sulfide stock solution.

### **314. NATRANAEROBACULUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g

NH<sub>4</sub>Cl 1.0 g

KCl 0.2 g

NaCl 16.0 g

Trace element solution *SL-11* (see below) 1.0 ml

Yeast extract 0.2 g

Na-resazurin solution (0.1% w/v) 0.5 ml

Na<sub>2</sub>CO<sub>3</sub> 68.0 g

NaHCO<sub>3</sub> 38.0 g

Ethanol 5.0 ml

Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g

Distilled water 1000.0 ml

*Trace element solution SL-11:*

Na<sub>2</sub>-EDTA × 2 H<sub>2</sub>O 5.2 g

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 1000.0 ml

Dissolve EDTA in 800.0 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000.0 ml.

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients except carbonate, bicarbonate, ethanol, vitamins and sulfide. Sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Add and dissolve carbonates and sulfide while gassing the head space only. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add ethanol and vitamins from sterile anoxic stock solution

prepared under 100% N<sub>2</sub> gas. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 9.5 – 10.0, if necessary.

### **315. FUCHSIELLA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 0.5 g  
KCl 0.2 g  
NaCl 60.0 g  
Na<sub>2</sub>CO<sub>3</sub> 68.3 g  
NaHCO<sub>3</sub> 38.3 g  
Trace element solution *SL-4* (see below) 1.0 ml  
Resazurin 0.5 mg  
Vitamin solution (see below) 10.0 ml  
Yeast extract 0.05 g  
Ethanol (50% v/v) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g  
NaNO<sub>3</sub> 0.85 g  
Distilled water 1000.0 ml  
*Trace element solution SL-4:*  
EDTA 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
Trace element solution *SL-6* (see below) 100.0 ml  
Distilled water 900.0 ml  
*Trace element solution SL-6:*  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 8.8- 9.5  
Dissolve ingredients except the sodium carbonate, sodium bicarbonate, ethanol, and sodium sulfide. Boil medium for a few minutes and cool to room temperature while flushing with N<sub>2</sub>. Add and dissolve sodium carbonate, sodium bicarbonate and sodium sulfide while gassing the head space only. Dispense and autoclave under N<sub>2</sub>. Before use add 10 ml/l (v/v) of 50% (v/v) ethanol solution (flushed and autoclaved under N<sub>2</sub>).

### **316. MELIORIBACTER MEDIUM**

Solution A:

NaCl 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 0.25 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 4.0 g  
Trace element solution (see below) 1.0 ml  
Vitamin solution (see below) 10.0 ml  
Yeast extract 2.0 g  
NaHCO<sub>3</sub> 0.1 g  
Distilled water 1000.0 ml  
pH 7.2 – 7.8

**Solution B:**

Na<sub>2</sub>S × 9 H<sub>2</sub>O 3.0 g  
Distilled water 100.0 ml  
Trace element solution:  
(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 784.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 143.6 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.6 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 1.8 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 238.0 mg  
NiSO<sub>4</sub>(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
conc. HCl 10.0 ml  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Autoclave and add the following component after autoclaving from stock solutions: sterile solution B Na<sub>2</sub>S (3%) end concentration 0.3% and filter-sterilized vitamin solution (see below) 0.1 ml /10 ml.

**317. ORNATILINEA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.14 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.15 g  
NH<sub>4</sub>Cl 0.54 g  
Trace element solution *SL-11* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 2.5 g  
Yeast extract 2.3 g



Vitamins solution (see below) 10.0 ml

D-Glucose 2.2 g

L-Cysteine-HCl  $\times$  H<sub>2</sub>O 0.25 g

Na<sub>2</sub>S  $\times$  9 H<sub>2</sub>O 0.25 g

Distilled water 1000.0 ml

*Trace element solution SL-11:*

Na<sub>2</sub>-EDTA  $\times$  2 H<sub>2</sub>O 5.2 g

FeCl<sub>2</sub>  $\times$  4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub>  $\times$  4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 36.0 mg

Distilled water 1000.0 ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride.

After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000.0 ml.

*Selenite-tungstate solution:*

NaOH 0.5 g

Na<sub>2</sub>SeO<sub>3</sub>  $\times$  5 H<sub>2</sub>O 3 mg

Na<sub>2</sub>WO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 4 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl  $\times$  2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, yeast extract, vitamins, glucose and reducing agents), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Add solid bicarbonate and adjust pH to 7.0. Dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration), glucose, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 7.0, if necessary.

### **318. TEPIDIBACILLUS MEDIUM**

Sea salts (SIGMA) 30.0 g

NaNO<sub>3</sub> 1.0 g

*Wolfe's* mineral elixier (see below) 2.0 ml

VOSO<sub>4</sub>  $\times$  5 H<sub>2</sub>O solution (0.01% w/v) 0.5 ml

Na-resazurin solution (0.1% w/v) 0.5 ml

FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.1 g

NaHCO<sub>3</sub> 1.0 g

Yeast extract 0.5 g

D-glucose 2.5 g

Vitamins solution (see below) 20.0 ml

Distilled water 1000.0 ml

*Wolfe's mineral elixir:*

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 30.0 g

MnSO<sub>4</sub> × H<sub>2</sub>O 5.0 g

NaCl 10.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 1.8 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1.8 g

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.1 g

KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.18 g

H<sub>3</sub>BO<sub>3</sub> 0.1 g

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 g

(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 2.8 g

Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.1 g

Na<sub>2</sub>SeO<sub>4</sub> 0.1 g

Distilled water 1000.0 ml First adjust pH to 1.0 with diluted H<sub>2</sub>SO<sub>4</sub>, then add and dissolve the salts.

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except ferrous sulfate, bicarbonate, yeast extract, D-glucose and vitamins), adjust pH to 7.0 and sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense the medium under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. Add ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Prepare the stock solution of ferrous sulfate by dissolving 1% (w/v) FeSO<sub>4</sub> × 7 H<sub>2</sub>O in 0.1 N H<sub>2</sub>SO<sub>4</sub>. The stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.8-7.0. Note: It may be necessary to add 10-20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under 100% N<sub>2</sub> and filter-sterilized), if the medium is not completely reduced after inoculation.

### **319. DEFERRISOMA MEDIUM**

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

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

KCl 0.33 g

NaCl 18.0 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.0 g

Yeast extract 0.2 g

Trace element solution *SL-10* (see below) 1.0 ml

Selenite-tungstate solution (see below) 1.0 ml

Fe(III)-citrate (19% Fe) 2.5 g

NaHCO<sub>3</sub> 2.5 g

Vitamin solution (see below) 10.0 ml

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except ferric citrate, bicarbonate and vitamins) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferric citrate (dissolve by boiling and adjust pH to 6.5 – 7.0) and vitamins from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Sterilize vitamins by filtration. Adjust pH of the complete medium to 6.5, if necessary.

**320. THERMOSULFURIMONAS MEDIUM**

NaCl 18.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.0 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
NaHCO<sub>3</sub> 2.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Vitamin solution (see below) 1.0 ml  
Na-acetate × 3 H<sub>2</sub>O 1.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O 3.5 g  
Yeast extract 0.2 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, phosphate, vitamins, acetate, thiosulfate and yeast extract, then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic.

Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add phosphate, vitamins, acetate, thiosulfate and yeast extract from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. The pH of the complete medium should be at 6.5 – 6.8.

**321. BROCKIA LITHOTROPHICA MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.44 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.7 g  
NaCl 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Sulfur, powdered 10.0 g  
NaHCO<sub>3</sub> 0.8 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4, and dispense medium under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins (sterilized by filtration) and sulfide are added to the medium from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 6.5, if necessary.

### **322. CLOSTRIDIUM MEDIUM II**

Ground beef (fat free) 500.0 g  
Distilled water 1000.0 ml  
NaOH 1 N 25.0 ml

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1000.0 ml, and then add:

Casitone 30.0 g  
Yeast extract 5.0 g  
K<sub>2</sub>HPO<sub>4</sub> 5.0 g  
Resazurin solution (0.1% w/v) 0.5 ml  
Glucose 4.0 g  
Cellobiose 1.0 g  
Maltose 1.0 g  
Starch (soluble) 1.0 g

To make medium anoxic boil it, cool under 100% N<sub>2</sub> gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under same gas atmosphere 7.0 ml medium into Hungate-type tubes.

### **323. PSYCHROPHYLIC CLOSTRIDIUM MEDIUM**

Tryptone 10.0 g  
Gelatin peptone 10.0 g  
Yeast extract 5.0 g

D-glucose 1.0 g  
Sodium chloride 5.0 g  
L-Arginine 1.0 g  
Sodium pyruvate 1.0 g  
Menadione 0.5 mg  
Haemin 5.0 mg  
Distilled water 990 ml

Dissolve all components in distilled water and add 0.5 ml/l Na-resazurin solution (0.1% w/v), bring to the boil and cool to room temperature while sparging with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add L-cysteine-HCl × H<sub>2</sub>O (0.3 g/l), dispense the medium into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving supplement medium with 5.0 g/l D-glucose added from a sterile anoxic stock solution sterilized by filtration. Adjust pH of medium to 6.8 with a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere.

### **324. SPHAEROCHAETA MEDIUM**

NaCl 1.0 g  
KCl 0.5 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 0.3 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 0.15 g  
Xylose 20 mmol  
Yeast extract 1.0 g  
NaHCO<sub>3</sub> 0.5 g  
Trace element solution *SL-7* (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml

Distilled water 990.0 ml

*Trace mineral solution:*

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.99 g  
CoCl<sub>2</sub> 0.13 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.198 g  
ZnCl<sub>2</sub> 0.136 g  
H<sub>3</sub>BO<sub>3</sub> 0.062 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.013 g  
AlCl<sub>3</sub> × 6 H<sub>2</sub>O 0.0133 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.0242 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.017 g  
Distilled water 1000.0 ml

*Seven vitamins solution:*

Vitamin B<sub>12</sub> 100.0 µg  
*p*-Aminobenzoic acid 80.0 mg  
D(+)-Biotin 20.0 mg  
Nicotinic acid 200.0 mg  
Calcium pantothenate 100.0 mg  
Pyridoxine hydrochloride 300.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 200.0 mg  
Distilled water 1000.0 ml

Dissolve all components in distilled water and add 0.5 ml/l Na-resazurin solution (0.1% w/v), bring to the boil and cool to room temperature while sparging with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add L-cysteine-HCl × H<sub>2</sub>O (0.3 g/l), dispense the medium into anoxic Hungate-type tubes or serum vials and autoclave. Sodium hydrogen carbonate, vitamins, xylose and antibiotics (when necessary) were added to the autoclaved medium from sterile anaerobic stock solutions. Adjust pH of medium to 7.2-7.3 with

a sterile anoxic stock solution of  $\text{Na}_2\text{CO}_3$  (5% w/v) prepared under 80%  $\text{N}_2$  and 20%  $\text{CO}_2$  gas atmosphere.

### **325. COPROBACTER MEDIUM**

Ground beef (fat free) 500.0 g

Distilled water 1000.0 ml

NaOH 1 N 25.0 ml

Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate.

To the filtrate add water to a final volume of 1000.0 ml, and then add:

Casitone 30.0 g

Yeast extract 5.0 g

$\text{K}_2\text{HPO}_4$  5.0 g

Resazurin 1.0 mg

To make medium anoxic bring it to a boil, cool under 100%  $\text{N}_2$  gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under 100%  $\text{N}_2$  gas atmosphere by filling 7 ml medium into anoxic Hungate-type tubes. Autoclave at 121°C for 15 min. Add to 1000.0 ml of medium after autoclaving: Haemin solution (see below) 10.0 ml Vitamin K1 or Vitamin K3 solution (see below) 0.2 ml.

Haemin solution: Dissolve 50 mg haemin in 1.0 ml 1 N NaOH; make up to 100.0 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K<sub>1</sub> solution: Dissolve 0.1 ml of vitamin K<sub>1</sub>/K<sub>3</sub> in 20.0 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K<sub>3</sub> solution: Dissolve 5 mg/ml of vitamin K<sub>3</sub> in 10.0 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

### **326. METHANOCALCULUS NATRONOPHILUS MEDIUM**

$\text{KH}_2\text{PO}_4$  0.2 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.1 g

$\text{NH}_4\text{Cl}$  1.0 g

KCl 0.2 g

NaCl 60.0 g

Trace element solution *SL-11* (see below) 1.0 ml

Na-acetate  $\times 3 \text{H}_2\text{O}$  0.2 g

Na-resazurin solution (0.1% w/v) 0.5 ml

$\text{Na}_2\text{CO}_3$  68.0 g

$\text{NaHCO}_3$  38.0 g

Vitamin solution (see below) 10.0 ml

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  1.0 g

Distilled water 1000.0 ml

*Trace element solution SL-11:*

$\text{Na}_2\text{-EDTA} \times 2 \text{H}_2\text{O}$  5.2 g

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.5 g

$\text{ZnCl}_2$  70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg

$\text{H}_3\text{BO}_3$  6.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  36.0 mg

Distilled water 1000.0 ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride.

After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000.0 ml.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl  $\times$  2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except carbonate, bicarbonate, vitamins and sulfide, then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Add and dissolve carbonates and sulfide while gassing the head space only. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add vitamins from an anoxic stock solution sterilized by filtration. Adjust pH of the complete medium to 9.0.

**327. THERMOANAEROBACTER SIDEROPHILUS MEDIUM**

Tryptone 1.0 g  
Peptone (meat) 1.0 g  
Yeast extract 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.6 g  
NaH<sub>2</sub>PO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 1.0 g  
NH<sub>4</sub>Cl 0.5 g  
MgSO<sub>4</sub>  $\times$  6 H<sub>2</sub>O 0.16 g  
Trace element solution *SL-11* (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.06 g  
NaHCO<sub>3</sub> 1.0 g  
Vitamin solution (see below) 10.0 ml  
L-Cysteine-HCl  $\times$  H<sub>2</sub>O 0.3 g  
Na<sub>2</sub>S  $\times$  9 H<sub>2</sub>O 0.3 g  
Distilled water 1000.0 ml

*Trace element solution SL-11:*

Na<sub>2</sub>-EDTA  $\times$  2 H<sub>2</sub>O 5.2 g  
FeCl<sub>2</sub>  $\times$  4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub>  $\times$  4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 36.0 mg  
Distilled water 1000.0 ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000.0 ml.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl  $\times$  2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg



Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.0 and sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N<sub>2</sub> gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

### **328. MOORELLA GLYCERINI MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
Yeast extract 0.5 g  
Glycerol (87%) 3.0 ml  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 10.0 g  
Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, vitamins and sulfide, then sparge medium with 100% CO<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dissolve bicarbonate and adjust pH to 6.7, then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins should be sterilized by filtration

### **329. *TEPIDIBACTER* MEDIUM**

NaCl 18.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.0 g

KCl 0.34 g

NH<sub>4</sub>Cl 0.25 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.11 g

K<sub>2</sub>HPO<sub>4</sub> 0.18 g

Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg

Trace element solution *SL-10* (see below) 1.0 ml

Selenite-tungstate solution (see below) 1.0 ml

Yeast extract 0.2 g

Proteose peptone 10.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

NaHCO<sub>3</sub> 5.0 g

Vitamin solution (see below) 10.0 ml

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

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g

Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg

Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. The pH of the complete medium should be 6.5 - 7.0.

### **330. CLOSTRIDIUM CELLULOLYTICUM MEDIUM**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.3 g  
KH<sub>2</sub>PO<sub>4</sub> 1.5 g  
K<sub>2</sub>HPO<sub>4</sub> × 3 H<sub>2</sub>O 2.9 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O solution (0.1% w/v in 0.1 N H<sub>2</sub>SO<sub>4</sub>) 1.25 ml  
Trace element solution *SL-10* (see below) 1.0 ml  
Yeast extract 2.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 75.0 mg  
Cellobiose 6.0 g  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>CO<sub>3</sub> 2.5 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

Dissolve ingredients except magnesium chloride, calcium chloride, cellobiose, cysteine and carbonate, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. After autoclaving add magnesium chloride, calcium chloride and cellobiose from anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Cellobiose has to be sterilized by filtration. Prior to inoculation add cysteine from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas and adjust pH to 7.2 by adding a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere.

### **331. THERMOCOCCUS STETTERI MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
KCl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Casitone 5.0 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Sulfur, powder 10.0 g  
NaCl 2.5 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g

Distilled water 1000.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except sulfur, vitamins and sulfide) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 5.7, dispense under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and sterilize by heating for 2-3 hours in a boiling water bath. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Prior to use adjust pH of complete medium to 6.5 with sterile, anaerobic NaHCO<sub>3</sub>-solution.

### **332. THERMOGUTTA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
NaCl 6.0  
Yeast extract 0.2 g  
Xanthan 5.0 g  
KNO<sub>3</sub> 2.0 g  
NaHCO<sub>3</sub> 2.0 g  
Trace element solution (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
*Trace element solution:*  
(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O (Mohr's salt) 784.0 mg  
CoCl<sub>2</sub> × H<sub>2</sub>O 238.0 mg  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 144.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg

Mohr's salt is dissolved firstly in concentrated HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

*Vitamin solution:*

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine 10.0 mg

Riboflavin 50.0 mg

Pantotenoic acid 50.0 mg

*p*-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B<sub>12</sub> 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

The media was boiled and cooled under the flow of oxygen-free gases (CO<sub>2</sub> or N<sub>2</sub>) to render the media anaerobic and finally heat-sterilized at 121°C for 60 min. No reducing agents were added.

### **333. MEDIUM FOR PSYCHROPHILIC *METHANOSARCINA***

K<sub>2</sub>HPO<sub>4</sub> 0.35 g

KH<sub>2</sub>PO<sub>4</sub> 0.23 g

NH<sub>4</sub>Cl 0.5 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.25 g

NaCl 2.25 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O solution (0.1% w/v in 0.1 H<sub>2</sub>SO<sub>4</sub>) 2.0 ml

Trace element solution *SL-10* (see below) 1.0 ml

Yeast extract 2.0 g

Casitone 2.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

NaHCO<sub>3</sub> 0.85 g

Vitamin solution (see below) 10.0 ml

Methanol 10.0 ml

L-Cysteine-HCl × H<sub>2</sub>O 0.3 g

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.3 g

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Then add and dissolve bicarbonate, adjust pH to 6.8 and dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N<sub>2</sub> gas atmosphere as concentrated solutions in tightly closed tubes. Vitamins are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Appropriate volumes of the solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.

### **334. METHANOSPIRILLUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.23 g  
K<sub>2</sub>HPO<sub>4</sub> 0.23 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.09 g  
NaCl 0.46 g  
NH<sub>4</sub>Cl 0.4 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 60.0 mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> × 7 H<sub>2</sub>O 0.23 g  
Trace element solution *SL-10* (see below) 10.0 ml  
Vitamin solution (see below) 10.0 ml  
Yeast extract 1.0 g  
Na-acetate × 3 H<sub>2</sub>O 1.0 g  
Na-formate 2.0 g  
Trypticase 0.5 g  
Casamino acids 0.2 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 4.0 g  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 980.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, cysteine and sulfide. Sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

### **335. CARNOBACTERIUM MEDIUM**

Trypticase soy broth 30.0 g  
Yeast extract 3.0 g  
NaCl 13.0 g  
KCl 0.34 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.45 g  
NH<sub>4</sub>Cl 0.25 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.14 g  
Distilled water 1000.0 ml  
Adjust pH to 7.

### **336. ALKALIPHILIC SPIROCHAETE MEDIUM**

Na<sub>2</sub>CO<sub>3</sub> 10.0 g  
NaHCO<sub>3</sub> 15.0 g  
NaCl 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 1.0 g  
KCl 0.2 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 1.0 g  
Yeast extract 0.5 g  
Sucrose 5.0 g  
Vitamin solution (see below) 10.0 ml  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Trace element solution:*

Nitilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg

Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl  $\times 2 \text{ H}_2\text{O}$  5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Prepare the medium under N<sub>2</sub>, omitting Na<sub>2</sub>CO<sub>3</sub>, NaHCO<sub>3</sub>, Na<sub>2</sub>S  $\times 9 \text{ H}_2\text{O}$ , sucrose and vitamin solution. Boil the medium and cool under nitrogen, add the Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> and adjust the pH to 9.7 with 6N NaOH (about 15 ml). Autoclave at 121°C for 15 min and add the Na<sub>2</sub>S  $\times 9 \text{ H}_2\text{O}$  (neutralized), vitamins and sucrose from sterile stock solutions.

### **337. CUNICULIPLASMA MEDIUM**

Beef extract 3 g  
Betaine 0.6 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.3 g  
KH<sub>2</sub>PO<sub>4</sub> 0.28 g  
MgSO<sub>4</sub>  $\times 7 \text{ H}_2\text{O}$  0.25 g  
CaCl<sub>2</sub>  $\times 2 \text{ H}_2\text{O}$  0.07 g  
FeCl<sub>3</sub>  $\times 6 \text{ H}_2\text{O}$  0.02 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Vitamin solution (see above) 10.0 ml  
Distilled water 990.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub>  $\times 4 \text{ H}_2\text{O}$  1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub>  $\times 4 \text{ H}_2\text{O}$  100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  190.0 mg  
CuCl<sub>2</sub>  $\times 2 \text{ H}_2\text{O}$  2.0 mg  
NiCl<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

*myo*-Inositol 1.0 g  
Thiamine-HCl 100.0 mg  
Pyridoxine-HCl 100.0 mg  
Folic acid 40.0 mg  
D-Biotin 10.0 mg  
L-Ascorbic acid 200.0 mg  
D-Ca-pantothenate 100.0 mg  
Choline chloride 100.0 mg  
Nicotinamide 100.0 mg  
Riboflavin 20.0 mg  
Vitamin B<sub>12</sub> 20.0 µg  
Vitamin A 10.0 mg  
*p*-Aminobenzoic acid 20.0 mg  
Distilled water 990.0 ml



The medium was adjusted to pH 1.0–1.2 with concentrated H<sub>2</sub>SO<sub>4</sub>. The cultures were incubated for about 5 days at 37°C with shaking.

### **338. MAGNETOSPIRILLUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.68 g  
NaNO<sub>3</sub> 0.12 g  
L(+)-Tartaric acid 0.37 g  
Succinic acid 0.37 g  
Na-acetate × 3 H<sub>2</sub>O 0.05 g  
Vitamin solution (see below) 10.0 ml  
Trace element solution (see below) 5.0 ml  
Fe(III) quinate solution (see below) 2.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Na-thioglycolate 0.05 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Ferric Quinate Solution, 0.01 M:*

FeCl<sub>3</sub> × 6 H<sub>2</sub>O 4.5 g  
Quinic acid 1.9 g  
Distilled water 1000.0 ml

Sterilize by filtration under 100% N<sub>2</sub> gas atmosphere.

Dissolve ingredients (except thioglycolate) and adjust pH to 6.75 with NaOH. Sparge medium with 100% N<sub>2</sub> gas for 30–45 min and dispense under the same gas atmosphere into anoxic Hungate – type tubes to 50% of their volume. Before inoculation add thioglycolate from a 0.5% (w/v) stock solution,

freshly prepared under 100% N<sub>2</sub> gas and filter-sterilized. Then add sterile air to a concentration of 2.5 % O<sub>2</sub> in the vial.

### 339. ACETOBACTERIUM MEDIUM

NH<sub>4</sub>Cl 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
K<sub>2</sub>HPO<sub>4</sub> 0.45 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
Trace element solution (see below) 20.0 ml  
Yeast extract 2.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 10.0 g  
D-Fructose 10.0 g  
Vitamin solution (see below) 10.0 ml  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine and sulfide, bring to the boil and cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add bicarbonate (solid) and equilibrate the medium with the gas until a pH of around 7.4 is reached. Then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Before use adjust the

pH to 8.0 - 8.2 by adding a sterile anoxic stock solution of sodium carbonate (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture (0.25 ml per 10.0 ml medium) and add fructose, vitamins (sterilized by filtration), cysteine and sulfide from anoxic sterile stock solutions prepared under 100% N<sub>2</sub>.

#### **340. DISSULFURIMICROBIUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O, 0.33 g  
NaHCO<sub>3</sub> 2.0 g  
Vitamin solution (see below) 10.0 ml  
Trace element solution (see below) 1.0 ml  
Ferrihydrite  
Distilled water 990.0 ml

##### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

##### *Trace element solution:*

Na<sub>2</sub>-EDTA × 2 H<sub>2</sub>O 5.2 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 1000.0 ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000.0 ml.

The pH of the autoclaved medium was 6.7–6.8.

Medium (10.0 ml) was dispensed into 17 ml Hungate tubes; the headspace was filled with CO<sub>2</sub> (100 %).

#### **341. RUTHENIBACTERIUM MEDIUM**

Trypticase peptone 5.0 g  
Peptone 5.0 g  
Yeast extract 10.0 g  
Beef extract 5.0 g  
Glucose 5.0 g

K<sub>2</sub>HPO<sub>4</sub> 2.0 g  
Tween 80 1.0 ml  
L-Cysteine-HCl × H<sub>2</sub>O 0.5 g  
Resazurin 1.0 mg  
Salt solution (see below) 40.0 ml  
Distilled water 950.0 ml  
Haemin solution (see below) 10.0 ml  
Vitamin K<sub>1</sub> solution (see below) 0.2 ml  
The vitamin K<sub>1</sub>, haemin solution and the cysteine are added after the medium has been boiled and cooled under CO<sub>2</sub>. Adjust pH to 7.2 using 8 N NaOH. Distribute under N<sub>2</sub> and autoclave.

Salt solution:

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.25 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
NaHCO<sub>3</sub> 10.0 g  
NaCl 2.0 g  
Distilled water 1000.0 ml

*Haemin solution:* Dissolve 50 mg haemin in 1.0 ml 1 N NaOH; make up to 100.0 ml with distilled water. Store refrigerated.

*Vitamin K<sub>1</sub> solution:* Dissolve 0.1 ml of vitamin K<sub>1</sub> in 20.0 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

### **342. RHODOVULUM MEDIUM**

Yeast extract 0.3 g  
Na<sub>2</sub>-succinate 1.0 g  
(NH<sub>4</sub>)-acetate 0.5 g  
Fe(III) citrate solution (0.1% in H<sub>2</sub>O) 5.0 ml  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.4 g  
NaCl 0.4 g  
NH<sub>4</sub>Cl 0.4 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
Vitamin B<sub>12</sub> solution (10 mg in 100 ml H<sub>2</sub>O) 0.4 ml  
Trace element solution *SL-6* (see below) 1.0 ml  
L-Cysteine chloride × H<sub>2</sub>O 0.3 g  
Resazurin (0,1%) 0.5 ml  
Distilled water 1000.0 ml

Adjust pH to 6.8. Boil the medium for a few minutes. Bubble the medium with nitrogen gas and fill 10.0 ml in tubes with a rubber septum under a stream of nitrogen gas. Autoclave at 121°C for 15 min. Sterile syringes are used to inoculate and remove samples. Incubate in the light using a tungsten lamp.

*Trace element solution SL-6:*

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

### **343. HALOMONAS MEDIUM**

NaCl 80.0 g  
Casamino acids 7.5 g

Proteose peptone 5.0 g  
Yeast extract 1.0 g  
Sodium citrate 3.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 0.05 g  
Distilled water 1000.0 ml  
Adjust pH to 7.0 with KOH.  
Autoclave at 121°C for 15 min.

#### **344. COLUMBIA AGAR**

Special mixture of peptones 23.0 g  
Starch 1.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.3±0.2.  
Adjust pH to 7.3±0.2. Autoclave at 121°C for 15 min.

#### **345. NATRONOSPIRILLUM SPERANDUS MEDIUM**

NaCl 34.0 g  
NaHCO<sub>3</sub> 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.3 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.12 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g  
Casamino acids 3.0 g  
Yeast extract 0.1 g  
Agar 20.0 g  
Trace element solution (see below) 10.0 ml  
Distilled water 1000.0 ml. Autoclave at 121°C for 15 min.  
Final pH 9.0 – 9.5

##### *Trace element solution:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.6 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.12 mg  
Ni(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 0.9 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.14 mg  
H<sub>3</sub>BO<sub>3</sub> 0.03 mg  
AlK(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.24 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.02 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 mg  
Na<sub>2</sub>SeO<sub>3</sub> 0.02 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg  
Distilled water 1000.0 ml

Autoclave the trace element solution and NaHCO<sub>3</sub> separately at 121°C for 15 min and add to the medium.

Final pH 8.7 – 8.9

#### **346. THERMAEROBACTER BAIKALENSIS MEDIUM**

Peptone 1.25 g  
Yeast extract 0.25 g  
Fe(III) citrate 0.025 g  
NaCl 5.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 1.0 g

Na<sub>2</sub>SO<sub>4</sub> 0.8 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.45 g  
KCl 0.1 g  
NaHCO<sub>3</sub> 0.4 g  
KBr 0.02 g  
SrCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.02 g  
Agar 20.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml. Autoclave at 121°C for 15 min.  
Final pH 7.0

*Trace element solution:*

Na-silicate 4.0 g  
NaF 2.4 g  
NH<sub>4</sub>NO<sub>3</sub> 1.6 g  
Na<sub>2</sub>HPO<sub>4</sub> 8.0 g  
Distilled water 1000.0 ml. Autoclave the trace element solution separately at 121°C for 15 min and add to the medium.

**347. SUCCINATE MINIMAL SALT MEDIUM (SMS)**

EDTA 0.01 g  
KH<sub>2</sub>PO<sub>4</sub> 0.6 g  
K<sub>2</sub>HPO<sub>4</sub> 0.9 g  
NH<sub>4</sub>Cl 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.075 g  
Na-succinate 2.2 g  
Yeast extract 0.1 g  
Trace elements solution (see below) 2.0 ml  
Vitamin solution (see below) 2.0 ml

Distilled water 1000.0 ml

*Trace elements solution:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 300.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 5.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 3.0 mg  
H<sub>3</sub>BO<sub>3</sub> 2.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 5.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 3.0 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 80.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 400.0 mg  
Nicotinic acid 400.0 mg  
Vitamin B<sub>12</sub> 20.0 µg  
Distilled water 1000.0 ml

Adjust pH to 6.8. Prepare the medium without the vitamin solution. Autoclave at 121°C for 15 min and add the vitamin solution from a filter-sterilized stock solution.

**348. RO (RICH ORGANIC) MEDIUM**

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.3 g  
KH<sub>2</sub>PO<sub>4</sub> 0.3 g  
NH<sub>4</sub>Cl 0.1 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 g  
Na-acetate × 3 H<sub>2</sub>O 1.0 g  
Yeast Extract 0.5 g  
Peptone 0.5 g  
Casamino Acid 0.5 g  
Trace elements solution (see below) 2.0 ml  
Vitamin solution (see below) 2.0 ml

Distilled water 1000.0 ml

*Trace elements solution:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 300.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 5.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 3.0 mg  
H<sub>3</sub>BO<sub>3</sub> 2.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 5.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 1.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 3.0 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 80.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 400.0 mg  
Nicotinic acid 400.0 mg  
Vitamin B<sub>12</sub> 20.0 µg  
Distilled water 1000.0 ml

Autoclave base medium, KH<sub>2</sub>PO<sub>4</sub> (in 10.0 ml distilled water) and trace element solution at 121°C for 15 min. Vitamin solution sterilize by filtration.

### **349. TAUTONGIA SOCIABILIS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
Xylose or trehalose 1.0 g  
Trace element solution (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
Distilled water 1000.0 ml

Autoclave base medium, KH<sub>2</sub>PO<sub>4</sub> (in 10.0 ml distilled water) and trace element solution at 121°C for 15 min. Vitamin solution sterilize by filtration. Adjust pH to 7.5-7.7.

*Trace element solution:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O (*Mohr's salt*) 784.0 mg  
HCl (concentrated) 5.0 ml  
CoCl<sub>2</sub> × H<sub>2</sub>O 238.0 mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>NiSO<sub>4</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 144.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg  
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with 1000.0 ml distilled water and other salts are dissolved in the sequence indicated.

*Vitamin solution:*

Biotin 20.0 mg  
Folic acid 20.0 mg  
Pyridoxine 100.0 mg  
Riboflavin 50.0 mg  
Pantotenoic acid 50.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Thiamine-HCl 50.0 mg  
Nicotinic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
Lipoic acid 50.0 mg  
Distilled water 1000.0 ml  
Adjust pH to 5.9.

After autoclaving at 121°C for 15 min add sterile stock solution NaOH (0.5 M, 3.2 ml/l medium) to readjust pH to 6.8. Add the vitamin solution and trace element solution from sterile stock solutions.

**350. THERMOGEMMATA POLYSACCHARIDOLYTICA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.165 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.165 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.165 g  
NH<sub>4</sub>Cl 0.165 g  
KCl 0.165 g  
Maltose or trehalose 1.0 g  
Trace element solution (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Trace element solution:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O (*Mohr's salt*) 784.0 mg  
HCl (concentrated) 5.0 ml  
CoCl<sub>2</sub> × H<sub>2</sub>O 238.0 mg  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 144.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg  
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

*Vitamin solution:*

Biotin 20.0 mg  
Folic acid 20.0 mg  
Pyridoxine 100.0 mg  
Riboflavin 50.0 mg  
Pantotenoic acid 50.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Thiamine-HCl 50.0 mg  
Nicotinic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
Lipoic acid 50.0 mg  
Distilled water 1000.0 ml



Autoclave base medium,  $\text{KH}_2\text{PO}_4$  (in 10.0 ml distilled water) and trace element solution at  $121^\circ\text{C}$  for 15 min. Vitamin solution sterilize by filtration. Adjust pH to 7.0-7.5.

### **351. PSEUDOALTEROMONAS SPIRALIS MEDIUM**

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.5 g  
 $\text{KH}_2\text{PO}_4$  0.3 g  
 $\text{NH}_4\text{Cl}$  0.3 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.05 g  
Na-acetate  $\times 3 \text{H}_2\text{O}$  1.0 g  
Yeast extract 0.5 g  
Casamino acids 0.5 g  
NaCl 15.0 g  
Trace elements solution (see below) 2.0 ml  
Vitamin solution (see below) 2.0 ml  
Distilled water 1000.0 ml

#### *Trace elements solution:*

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  0.3 g  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.03 g  
Distilled water 1000.0 ml

#### *Vitamin solution:*

Biotin 80 mg  
Thiamine-HCl 400.0 mg  
Nicotinic acid 400.0 mg  
Vitamin  $\text{B}_{12}$  20.0  $\mu\text{g}$   
Distilled water 1000.0 ml

After autoclaving at  $121^\circ\text{C}$  for 15 min adjust to pH 7.8 with  $\text{NaHCO}_3$ . Add the vitamin solution and trace elements solution from sterile stock solutions.

### **352. THERMUS MEDIUM**

Bacto Yeast Extract (Difco) 1.0 g  
Bacto Tryptone (Difco) 1.0 g  
Na- glutamate  $\times \text{H}_2\text{O}$  1.0 g  
 $\text{CaSO}_4 \times 2 \text{H}_2\text{O}$  0.06 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g  
NaCl 0.08 g  
 $\text{KNO}_3$  0.1 g  
 $\text{NaNO}_3$  0.69 g  
 $\text{Na}_2\text{HPO}_4$  0.11 g  
Trace element solution (see below) 10.0 ml  
Agar, if necessary 20.0 g  
Distilled water to 1000.0 ml  
Adjust pH to 7.5 with NaOH. Autoclave at  $121^\circ\text{C}$  for 15 min.

#### *Trace elements solution:*

Nitrilotriacetic acid 12.8 g  
 $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.35 g  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  0.1 g  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  0.024 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g  
 $\text{ZnCl}_2$  0.1 g  
 $\text{CuCl}_2$  0.025 g  
 $\text{H}_3\text{BO}_3$  0.01 g  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  0.024 g  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.12 g  
 $\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  0.04 g

Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with NaOH, and then add minerals. Final pH 7.0.

### **353. ALCALIPHILIC AMPHIBACILLUS MEDIUM**

$\text{KH}_2\text{PO}_4$  0.2 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.1 g

$\text{NH}_4\text{Cl}$  0.5 g

KCl 0.2 g

$\text{Na}_2\text{CO}_3$  63.6 g

$\text{NaHCO}_3$  50.4 g

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.7 g

Yeast extract 0.2 g

Sucrose 5.0 g

Trace elements (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Resazurin 0.01 g

Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  3.0 g

$\text{MnSO}_4 \times \text{H}_2\text{O}$  0.5 g

NaCl 1.0 g

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g

$\text{CoSO}_4 \times 7 \text{H}_2\text{O}$  0.18 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.18 g

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$  0.01 g

$\text{KAl}(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$  0.02 g

$\text{H}_3\text{BO}_3$  0.01 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  0.01 g

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.03 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  0.3 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$  0.4 mg

Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl  $\times 2 \text{H}_2\text{O}$  5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

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

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Final pH 9.5-10.0. Prepare the medium without adding the vitamins, sucrose, yeast extract,  $\text{NH}_4\text{Cl}$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{NaHCO}_3$  using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the  $\text{NH}_4\text{Cl}$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{NaHCO}_3$  after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave, and to the cooled medium add the vitamins, sucrose, yeast extract, and  $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  from anaerobic, sterile

stock solutions.

### **354. BACTO MARINE BROTH (DIFCO 2216)**

Bacto peptone 5.0 g

Bacto yeast extract 1.0 g

Fe(III) citrate 0.1 g

NaCl 80.0 g

MgCl<sub>2</sub> (anhydrous) 5.9 g

Na<sub>2</sub>SO<sub>4</sub> 3.24 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 1.8 g

KCl 0.55 g

NaHCO<sub>3</sub> 0.16 g

KBr 0.08 g

SrCl<sub>2</sub> 34.0 mg

H<sub>3</sub>BO<sub>3</sub> 22.0 mg

Na-silicate 4.0 mg

NaF 2.4 mg

NH<sub>4</sub>NO<sub>3</sub> 1.6 mg

Na<sub>2</sub>HPO<sub>4</sub> 8.0 mg

Distilled water 1000.0 ml C.

Final pH should be 7.6 ± 0.2 at 25

If using the complete medium from Difco add 37.4 g per liter water + 6% NaCl.

### **355. CLOSTRIDIUM PYG MEDIUM**

Trypticase peptone 5.0 g

Peptone from meat (pepsin-digested) 5.0 g

Yeast extract 10.0 g

Salt solution (see below) 40.0 ml

Na-resazurin solution (0.1% w/v) 0.5 ml

L-Cysteine-HCl × H<sub>2</sub>O 0.5 g

Na<sub>2</sub>CO<sub>3</sub> 2.5 g

D-Glucose 5.0 g

Distilled water 1000.0 ml

*Salt solution:*

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.25 g

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

K<sub>2</sub>HPO<sub>4</sub> 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

NaHCO<sub>3</sub> 10.0 g

NaCl 2.0 g

Distilled water 1000.0 ml

Dissolve ingredients (except cysteine, carbonate and glucose) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Add cysteine, then dispense under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 7.0, if necessary.

### **356. ANOYXNATRONUM MEDIUM**

Na<sub>2</sub>CO<sub>3</sub> 25.0 g

NaHCO<sub>3</sub> 25.0 g

KCl 0.2 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g

NH<sub>4</sub>Cl 0.5 g

K<sub>2</sub>HPO<sub>4</sub> 0.2 g  
Yeast extract 0.2 g  
Trace elements *SL-10* (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.7 g  
Glucose 5.0 g  
Distilled water 1000.0 ml, pH 9.0

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Prepare the medium anaerobically without the vitamins, Na<sub>2</sub>S × 9 H<sub>2</sub>O, yeast extract, NaHCO<sub>3</sub>, and Na<sub>2</sub>CO<sub>3</sub>. Boil the medium, cool under N<sub>2</sub>, add the NaHCO<sub>3</sub>, and Na<sub>2</sub>CO<sub>3</sub>, dispense under N<sub>2</sub> and autoclave the medium. Add the vitamin solution, yeast extract, Na<sub>2</sub>S × 9 H<sub>2</sub>O and glucose from sterile stock solution prepared under N<sub>2</sub> to the cooled, autoclaved medium.

**357. THERMOLITHOBACTER MEDIUM**

KCl 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.52 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.29 g  
NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Trace element solution *SL-11* (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 1.0 g  
Yeast extract 0.05 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.7 g  
Distilled water 1000.0 ml

*Trace element solution SL-11:*

Na<sub>2</sub>-EDTA × 2 H<sub>2</sub>O 5.2 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 1000.0 ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000.0 ml.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, yeast extract, vitamins and sulfide, then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. After completing the medium adjust pH to 6.8-7.0. Inoculated vessels are pressurized with sterile carbon monoxide gas to 2 bar overpressure.

**358. THERMOANAEROBACTERIUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.3 g  
Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 5.3 g  
NH<sub>4</sub>Cl 1.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Trace elements solution (see below) 10.0 ml  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O solution (0.1% w/v in 0.1 N H<sub>2</sub>SO<sub>4</sub>) 1.5 ml  
Yeast extract 1.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
D-Glucose 5.0 g  
Vitamin solution (see below) 5.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitilotriacetic acid (NTA) 12.8 g  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 0.2 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.17 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnCl<sub>2</sub> 0.1 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
NaCl 1.0 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.03 g

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.0 ml.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl  $\times 2 \text{ H}_2\text{O}$  5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except glucose, vitamins and sulfide), adjust pH to 6.0, sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. After sterilization add glucose and sulfide from anoxic stock solutions autoclaved under 100% N<sub>2</sub> gas and vitamins from a filter-sterilized anoxic stock solution prepared under 100% N<sub>2</sub>. Adjust pH of complete medium to 6.0 – 6.5, if necessary.

**359. DESULFOVERMICULUS MEDIUM**

NaCl 100.0 g  
MgSO<sub>4</sub>  $\times 7 \text{ H}_2\text{O}$  10.0 g  
KCl 6.0 g  
CaCl<sub>2</sub>  $\times 2 \text{ H}_2\text{O}$  0.4 g  
NH<sub>4</sub>Cl 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
Yeast extract 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
NaHCO<sub>3</sub> 4.0 g  
Na-(DL)-malate 1.0 g  
Resazurin 0.5 mg  
Na<sub>2</sub>S  $\times 9 \text{ H}_2\text{O}$  0.3 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub>  $\times 4 \text{ H}_2\text{O}$  1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub>  $\times 4 \text{ H}_2\text{O}$  100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  190.0 mg  
CuCl<sub>2</sub>  $\times 2 \text{ H}_2\text{O}$  2.0 mg  
NiCl<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub>  $\times 5 \text{ H}_2\text{O}$  3 mg  
Na<sub>2</sub>WO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  4 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except lactate, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Dispense under same gas atmosphere in culture vessels and autoclave at 121°C 15 min. Add sodium lactate and sulfide from sterile anoxic

stock solutions prepared under N<sub>2</sub> and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> (all solutions sterilize separately at 121°C 15 min).

Final pH of the medium 7.0-7.2.

### **360. METHANOSARCINA MEDIUM**

NaCl 5.0 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g

NH<sub>4</sub>Cl 1.0 g

Casamino acids 1.0 g

Resazurin 0.01 g

Methanol 10% 10.0 ml

Trace element solution (see below) 10.0 ml

Vitamin solution (see below) 5.0 ml

L-Cysteine chloride × H<sub>2</sub>O 0.5 g

Distilled water 965.0 ml

*Buffer solutions:*

a) K<sub>2</sub>HPO<sub>4</sub> 29.0 g

Distilled water 100.0 ml

b) KH<sub>2</sub>PO<sub>4</sub> 15.0 g

Distilled water 100.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg

CoCl<sub>2</sub> × 2 H<sub>2</sub>O 0.17 mg

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 mg

ZnCl<sub>2</sub> 0.1 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg

H<sub>3</sub>BO<sub>3</sub> 0.01 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 mg

NaCl 1.0 mg

Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 10.0 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

*p*-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

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

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization.

Solutions of buffer (per 1 ml) add to base medium after separate sterilization. Base medium, trace element and buffer solutions autoclave at 121°C for 15 min. Vitamin solution is filter sterilized.

### **361. METHANOBACTERIUM ARCTICUM MEDIUM**

*Solution 1:*

KCl 0.34 g

MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.4 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.35 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 0.25 g  
KH<sub>2</sub> PO<sub>4</sub> 0.14 g  
NaCl 5.0 g  
Resazurin 0.01 g  
NaHCO<sub>3</sub> 5.0 g  
Trace element solution (see below) 10.0 ml  
Distilled water 965.0 ml

*Solution 2 (reducing agents):*

L-Cysteine chloride × H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> 0.18 g  
CuSO<sub>4</sub> 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.025 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Distilled water 1000.0 ml  
pH 7.0-7.4.

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization. Solutions of reducing agents (10.0 ml) and others solutions add to base medium after separate sterilization at 121°C for 15 min. Sterilize vitamin solution by filtration. Cultivate in a gas mixture of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

### **362. GEOALKALIBACTER MEDIUM**

NH<sub>4</sub>Cl 0.5 g  
KCl 0.2 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NaCl 1.0 g  
Yeast extract 0.1 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Na<sub>2</sub>CO<sub>3</sub> 3.0 g  
NaHCO<sub>3</sub> 10.0 g  
Sulfur, powdered 10.0 g  
Na-acetate × 3 H<sub>2</sub>O 2.5 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g



ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except carbonates, sulfur and acetate), then sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Add solid carbonate and bicarbonate, adjust pH to 9.0 - 9.2, dispense under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a water bath to 90 – 100°C for 1 – 2 hours on each of 3 successive days. Add acetate from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas.

### **363. ANOXYBACILLUS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.1 g  
KCl 0.2 g  
NH<sub>4</sub>Cl 1.0 g  
NaCl 5.0 g  
Trace element solution *SL-10* (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Na<sub>2</sub>CO<sub>3</sub> 2.76 g  
NaHCO<sub>3</sub> 10.0 g  
Yeast extract 0.5 g  
D-Glucose 5.0 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve all ingredients except carbonates, yeast extract, glucose, vitamins and sulfide, then sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Add carbonate and hydrogencarbonate, adjust pH to 9.0 - 9.5, dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 9.5 – 9.7.

### **364. CALDANAEROBACTER MEDIUM**

KCl 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.52 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.29 g  
NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Trace element solution *SL-4* (see below) 10.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 2.5 g  
D-glucose 2.0 g  
Yeast extract 0.05 g  
Vitamin solution 1 (see below) 9.0 ml  
Seven vitamins solution (see below) 1.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.3 g  
Distilled water 1000.0 ml  
*Trace element solution SL-4:*

Na<sub>2</sub>-EDTA 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.

*Vitamin solution 1:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Seven vitamins solution:*

Vitamin B<sub>12</sub> 100.0 mg  
*p*-Aminobenzoic acid 80.0 mg

D(+)-Biotin 20.0 mg  
Nicotinic acid 200.0 mg  
D-Ca-pantothenate 100.0 mg  
Pyridoxine-HCl 300.0 mg  
Thiamine-HCl  $\times$  2 H<sub>2</sub>O 200.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, D-glucose, yeast extract, vitamins and sulfide, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30-45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

Add yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Stock solutions of pyruvate and vitamins are sterilized by filtration. After completing the medium adjust pH to 6.8-7.0 with a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> (5% w/v), if necessary.

### **365. THERMOANAEROBACTER MEDIUM II**

NH<sub>4</sub>Cl 1.0 g  
NaCl 0.1 g  
MgCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.05 g  
K<sub>2</sub>HPO<sub>4</sub>  $\times$  3 H<sub>2</sub>O 0.4 g  
Trace element solution (see below) 10.0 ml  
Yeast extract 0.75 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 2.6 g  
Cellobiose 4.0 g  
Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S  $\times$  9 H<sub>2</sub>O 0.25 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub>  $\times$  H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub>  $\times$  5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub>  $\times$  12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub>  $\times$  6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub>  $\times$  5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl  $\times$  2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg

Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, cellobiose, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cellobiose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Stock solutions of cellobiose and vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

### **366. *DESULFUROCOCCUS KAMCHATKENSIS* MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.44 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.7 g  
NaCl 0.5 g  
Trace element solution *SL-10* (see below) 1.0 ml

Yeast extract (OXOID) 0.2 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Sulfur, powdered 10.0 g  
D-Glucose 2.5 g  
Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except sulfur, glucose, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Adjust pH to 6.2 - 6.4 and dispense

medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

### **367. ANAEROBRANCA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
Na<sub>2</sub>HPO<sub>4</sub> × 2 H<sub>2</sub>O 3.9 g  
KCl 0.5 g  
Yeast extract 5.0 g  
Trace element solution (see below) 5.0 ml  
Na<sub>2</sub>-fumarate 1.5 g  
Vitamin solution (see below) 10.0 ml  
L-Cysteine-HCl × H<sub>2</sub>O 0.15 g

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.15 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except fumarate, vitamins, cysteine and sulfide), adjust pH to 8.5 and sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add fumarate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Stock solutions of fumarate and vitamins are sterilized by filtration. Adjust pH of complete medium to 8.5, if necessary.

### 368. METHANOBACTERIUM VETERUM MEDIUM

#### *Solution 1:*

Na-acetate  $\times$  3 H<sub>2</sub>O 0.5 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.45 g  
K<sub>2</sub>HPO<sub>4</sub> 0.29 g  
KH<sub>2</sub>PO<sub>4</sub> 0.18 g  
MgSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.12g  
CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.06 g  
NaCl 0.05  
Resazurin 0.01 g  
Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
Trace element solution (see below) 10.0 ml  
Vitamin solution (see below) 10.0 ml  
Distilled water 965.0 ml

#### *Solution 2 (reducing agents):*

L-Cysteine-HCl  $\times$  H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S  $\times$  9 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

#### *Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> 0.18 g  
CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> 0.18 g  
CuSO<sub>4</sub> 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub>  $\times$  12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> 0.025 g  
Na<sub>2</sub>SeO<sub>3</sub> 0.3 mg  
Distilled water 1000.0 ml  
pH 7.0-7.4

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization. Solutions of reducing agents (10.0 ml) and others solutions add to base medium after separate sterilization at 121°C for 15 min. Sterilize vitamin solution by filtration. Cultivate in a gas mixture of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

### 369. *CLOSTRIDIUM TEPIDIPROFUNDI* MEDIUM

NaCl 18.0 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 4.0 g  
KCl 0.34 g  
NH<sub>4</sub>Cl 0.25 g  
CaCl<sub>2</sub> 0.11 g  
K<sub>2</sub>HPO<sub>4</sub> 0.18 g  
Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg  
Trace element solution *SL-10* (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.0 ml  
Yeast extract 0.2 g  
Proteose peptone 10.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
NaHCO<sub>3</sub> 5.0 g  
Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. The pH of the complete medium should be 6.5 - 7.0.

### 370. *ACIDILOBUS* MEDIUM

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

KCl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
Trace mineral solution *SL-10* (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Sulfur, powdered 10.0 g  
Yeast extract 0.1 g  
D-glucose 2.0 g  
Vitamin solution (see below) 10.0 ml

Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.45 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients except D-glucose, sulfur, yeast extract, vitamins and sulfide. Adjust pH to 3.5 with H<sub>2</sub>SO<sub>4</sub> and sparge medium with 100% CO<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of sulfur. Heat vessels containing medium to 90°C for 1 hour on each of 3 successive days. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The pH of the complete medium should be at 3.5 - 3.8.

### **371. MINERAL MEDIUM “P” FOR METHANOTROPHIC BACTERIA**

Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O 1.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.7 g  
KNO<sub>3</sub> 1.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g  
NaCl 30.0 g  
NaHCO<sub>3</sub> 4.2 g  
Na<sub>2</sub>CO<sub>3</sub> 0.05 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Trace elements solution:*



EDTA 5.0 g  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

Prepare the medium without the NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>. Basal medium, trace element solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> sterilize separately at 121°C for 15 min. When preparing liquid media cool the mineral salts solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool the NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> stock solution and agar to 50-55°C before mixing.

The pH of the complete medium should be at 9.0.

### **372. PLATE COUNT AGAR WITH 1% NaCl**

Tryptone 5.0 g  
Yeast extract 2.5 g  
Glucose 1.0 g  
NaCl 10.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.0±0.2  
Autoclave at 121°C for 15 min.

### **373. MARINE AMMONIUM MINERAL SALTS**

Solution A:

NaCl 20.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.02 g  
Methanol 5.0 ml  
Trace element solution SL-10 (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
Distilled water 900.0 ml  
Adjust the pH to 7.2 if needed with NaOH/HCl

Solution B:

KH<sub>2</sub>PO<sub>4</sub> 0.36 g  
K<sub>2</sub>HPO<sub>4</sub> 2.34 g  
Distilled water 100.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Autoclave solution A, B and trace element solution separately at 121°C for 15 min and combine after cooling. Add filter sterilize methanol and vitamin solution.

**374. AMS MEDIUM**

Phosphate buffer:

KH<sub>2</sub>PO<sub>4</sub> 0.54 g  
K<sub>2</sub>HPO<sub>4</sub> 0.7 g  
Agar (if needed) 15.0 g  
Distilled water 600.0 ml

Salt solution:

NH<sub>4</sub>Cl 0.5 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.2 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 1.0 g  
FeSO<sub>4</sub> solution (see below) 80 µl  
Trace element solution (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
Distilled water 400.0 ml

FeSO<sub>4</sub> solution:

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
HCl (5 M) few drops  
Distilled water 10.0 ml

*Trace element solution*

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 60.0 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 10.0 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Distilled water 100.0 ml

Autoclave phosphate buffer, salt solution and trace element solution separately at 121°C for 15 min and mix before use. Add filter sterilize FeSO<sub>4</sub> solution and vitamin solution.

pH 6.8.

**375. METHYLOMICROBIUM BURYATENSE MEDIUM**

$\text{KH}_2\text{PO}_4 \times 12 \text{H}_2\text{O}$  0.5 g  
KNO<sub>3</sub> 0.5 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.4 g  
NaCl 5.0 g  
NaHCO<sub>3</sub> (1M) 25.0 ml  
Na<sub>2</sub>CO<sub>3</sub> (1M) 5.0 ml  
Distilled water 1000.0 ml  
pH 9.0–9.5  
Trace element solution (see below) 1 ml

*Trace elements solution:*

EDTA 5.0 g  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  2.0 g  
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  0.02 g  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  0.2 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

Prepare the medium without the NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>. Basal medium, trace element, NaHCO<sub>3</sub> (1M) and Na<sub>2</sub>CO<sub>3</sub> (1M) sterilize separately at 121°C 15 min. When preparing liquid media cool the mineral salts solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> solutions to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> stock solutions and agar to 50-55°C before mixing.

### **376. MINERAL MEDIUM WITH GELATIN**

NaCl 10.0 g  
 $\text{KH}_2\text{PO}_4$  0.14 g  
KCl 0.36 g  
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.4 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.05 g  
NH<sub>4</sub>Cl 0.25 g  
3-(*N*-Morpholino) propanesulfonic acid (MOPS) buffer 2.09 g  
Gelatin 1.0 g  
Distilled water 1000.0 ml  
Adjust the pH to 7.5 if needed with NaOH/HCl. Autoclave at 121°C for 15 min.

### **377. CHLOROFLEXUS MEDIUM**

$\text{KH}_2\text{PO}_4$  0.5 g  
NH<sub>4</sub>Cl 0.5 g  
 $\text{MgCl}_2$  0.3 g  
KCl 0.5 g  
NaCl 5.0 g  
 $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{H}_2\text{O}$  0.1 g  
Yeast extract 1.0 g  
Na-acetate  $\times 3 \text{H}_2\text{O}$  0.5 g  
Na-malate 0.5 g  
HEPES 3.0 g  
Trace element solution according to *Pfennig* (see below) 1.0 ml  
Vitamin solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Trace element solution according to Pfennig:*  
EDTA 1.5 g  
Trace element solution according to *Hogland* (see below) 6.0 ml  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.2 g

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.5 mg

*p*-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 100.0 ml

After autoclaving at 121°C for 15 min, add 1 ml of 5% Na<sub>2</sub>S × 9 H<sub>2</sub>O solution (autoclaved), 1 ml of 5% CaCl<sub>2</sub> × 2 H<sub>2</sub>O solution (autoclaved) and 10 ml of 3% NaHCO<sub>3</sub> solution (filter-sterilized).

Vitamin solution is filter sterilized. Adjust pH to 7.5.

### **378. POLYMORPHOSOMA TUNDRAE MEDIUM**

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.8 g

NH<sub>4</sub>NO<sub>3</sub> 0.1 g

KH<sub>2</sub>PO<sub>4</sub> 0.04 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 g

Fructose 0.5 g

Yeast extract 0.01 g

Pectin 0.05 g

Phytigel 9.0 g

Distilled water 1000.0 ml

Adjust to pH 4.9 – 5.5. Autoclave at 121°C for 15 min.

### **379. GRANULICELLA SIBIRICA MEDIUM**

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.05 g

NH<sub>4</sub>NO<sub>3</sub> 0.1 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g

Glucose 0.5 g

Yeast extract 0.25 g

*Staley's* vitamin solution (see below) 1.0 ml

Distilled water 1000.0 ml

Adjust pH to 5.0-5.5

*Staley's Vitamin Solution:*

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

Biotin 2.0 mg

Thiamine-HCl  $\times$  2 H<sub>2</sub>O 5.0 mg  
Ca-pantothenate 5.0 mg  
Folic acid 2.0 mg  
Riboflavin 5.0 mg  
Nicotinamide 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Pyridoxine hydrochloride 10.0 mg  
Distilled water 1000.0 ml.  
Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution.

### **380. LIMNOGLOBUS ROSEUS MEDIUM**

Peptone 0.1 g  
Yeast extract 0.25 g  
NH<sub>4</sub>NO<sub>3</sub> 0.1 g  
Glucose 0.5 g  
*Hutner's* basal salts 20.0 ml  
*Staley's* vitamin solution, double concentration (see below) 1.0 ml  
Phytigel 10.0 g  
Distilled water 1000.0 ml  
Adjust pH to 6.5.  
*Hutner's basal salts*  
Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub>  $\times$  2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub>  $\times$  2 H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 99.0 mg  
Metal salt solution "44" (see below) 50.0 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Adjust volume to 1000.0 ml with distilled water.

#### *Metal solution "44":*

Na-EDTA 250.0 mg  
ZnSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub>  $\times$  7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub>  $\times$  5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub>  $\times$  6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>  $\times$  10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

#### *Staley's vitamin solution, double concentration:*

Vitamin B<sub>12</sub> 0.2 mg  
Biotin 4.0 mg  
Thiamine-HCl  $\times$  2 H<sub>2</sub>O 10.0 mg  
Ca-pantothenate 10.0 mg  
Folic acid 4.0 mg  
Riboflavin 10.0 mg  
Nicotinamide 10.0 mg  
*p*-Aminobenzoic acid 10.0 mg  
Pyridoxine hydrochloride 20.0 mg  
Distilled water 1000.0 ml.  
Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution.

### 381. *ALKALICAULIS SATELLES* MEDIUM

NaCl 42.0 g  
NaHCO<sub>3</sub> 24.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.3 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.12 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.03 g  
Trypton 2.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml

#### *Trace element solution:*

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.6 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 0.12 mg  
Ni (NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 0.9 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.1 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.14 mg  
H<sub>3</sub>BO<sub>3</sub> 0.03 mg  
AlK(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.24 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.02 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.03 mg  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.02 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 0.02 mg  
Distilled water 1000.0 ml

Prepare the medium without NaHCO<sub>3</sub>. Basal medium, trace element solution and NaHCO<sub>3</sub> sterilize separately at 121°C for 15 min. When preparing liquid media cool the mineral salts solution and NaHCO<sub>3</sub> to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool the NaHCO<sub>3</sub> stock solution and agar to 50-55°C before mixing.  
pH 8.0-9.0

### 382. *VIBRIO HARVEYI* MEDIUM

NaCl 30.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
Na<sub>2</sub>HPO<sub>4</sub> 6.0 g  
(NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Peptone 5.0 g  
Glycerol 3.0 ml  
Distilled water 1000.0 ml  
Autoclave at 121°C 15 min.

### 383. MEDIUM FOR MARINE PLANCTOMYCETES

*Hutner's* basal salts medium (see below) 20.0 ml  
Peptone Bacto 1.0 g  
Yeast extract Bacto 1.0 g  
HEPES 2.38 g  
Artificial seawater (see below) 250.0 ml  
Distilled water 690.0 ml (less for plates)  
Adjust pH to 7.5 with 5 M KOH  
After autoclaving and cooling (please see below for plates) add to the medium:  
Glucose solution (25%, sterile-filtered) 4.0 ml  
Vitamin solution 5.0 ml  
N-Acetylglucosamine 20.0 ml of 50.0 g/l stock solution  
Trace element solution 1.0 ml  
*Artificial sea water:*  
NaCl 46.94 g

Na<sub>2</sub>SO<sub>4</sub> 7.84 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 21.28 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 2.86 g  
NaHCO<sub>3</sub> 0.384 g  
KCl 1.384 g  
KBr 0.192 g  
H<sub>3</sub>BO<sub>3</sub> 0.052 g  
SrCl<sub>2</sub> × 6 H<sub>2</sub>O 0.08 g  
NaF 0.06 g

Distilled water 1000.0 ml

Do not autoclave. Due to high salt concentration, no filtration. Sometimes precipitation after a while or fungal growth observed, thus better prepare fresh. Store at room temperature.

*Hutner's basal salts medium:*

Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01267 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.099 g

Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).

Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

*Metal solution "44":*

Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

*Vitamin solution:*

Biotin 4.0 mg  
Folic acid 4.0 mg  
Pyridoxine-HCl 20.0 mg  
Riboflavine 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 10.0 mg  
Nicotinamide 10.0 mg  
D-Ca-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 0.2 mg  
*p*-Aminobenzoic acid 10.0 mg  
Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

*Trace element solution:*

Na-Nitrilotriacetat 1.5 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 500.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 100.0 mg  
ZnCl<sub>2</sub> 100.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 50.0 mg  
H<sub>2</sub>SeO<sub>3</sub> 50 mg

CuSO<sub>4</sub> × 5 H<sub>2</sub>O 10.0 mg  
AlK(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 10.0 mg  
H<sub>3</sub>BO<sub>3</sub> 10.0 mg  
NaMoO<sub>4</sub> × 2 H<sub>2</sub>O 10.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 10.0 mg  
Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

15.0 g/l Agar (Bacto) 3x washed with distilled water, autoclave separately and take volume of distilled water into account.

or

8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

### **384. MEDIUM FOR LIMNIC PLANCTOMYCETES**

NH<sub>4</sub>Cl 0.53 mg  
KH<sub>2</sub>PO<sub>4</sub> 1.4 mg  
KNO<sub>3</sub> 10 mg  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 49.3 mg  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 14.7 mg  
CaCO<sub>3</sub> 25 mg  
NaHCO<sub>3</sub> 25 mg  
Peptone Bacto 1.0 g  
Yeast extract Bacto 1.0 g  
HEPES 2.38 g  
*Hutner's* basal salts medium (see below) 20.0 ml  
Distilled water 950 ml (less for plates)  
Adjust pH to 7.0 with 5 M KOH  
After autoclaving and cooling (please see below for plates) add to the medium:  
Glucose solution (25%, sterile-filtered) 4.0 ml  
Vitamin solution 5.0 ml  
N-Acetylglucosamine 20.0 ml of 50.0 g/l stock solution  
Trace element solution 1.0 ml

*Hutner's basal salts medium:*

Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01267 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.099 g  
Metal salt solution "44" (see below) 50.0 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).

Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

*Metal solution "44":*

Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.



Sterilize by filtration, store at +4°C.

*Vitamin solution:*

Biotin 4.0 mg

Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg

Riboflavine 10.0 mg

Thiamine-HCl  $\times 2 \text{ H}_2\text{O}$  10.0 mg

Nicotinamide 10.0 mg

D-Ca-pantothenate 10.0 mg

Vitamin B<sub>12</sub> 0.2 mg

*p*-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

*Trace element solution:*

Na-Nitrilotriacetate 1.5 g

MnSO<sub>4</sub>  $\times \text{H}_2\text{O}$  500.0 mg

FeSO<sub>4</sub>  $\times 7 \text{ H}_2\text{O}$  100.0 mg

Co(NO<sub>3</sub>)<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  100.0 mg

ZnCl<sub>2</sub> 100.0 mg

NiCl<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  50.0 mg

H<sub>2</sub>SeO<sub>3</sub> 50 mg

CuSO<sub>4</sub>  $\times 5 \text{ H}_2\text{O}$  10.0 mg

AlK(SO<sub>4</sub>)<sub>2</sub>  $\times 12 \text{ H}_2\text{O}$  10.0 mg

H<sub>3</sub>BO<sub>3</sub> 10.0 mg

NaMoO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  10.0 mg

Na<sub>2</sub>WO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

15.0 g/l Agar (Bacto) 3x washed with distilled water, autoclave separately and take volume of distilled water into account.

or

8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

### **385. MEDIUM SM**

KH<sub>2</sub>PO<sub>4</sub> 0.14 g

MgCl<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  0.4 g

CaCl<sub>2</sub>  $\times 2 \text{ H}_2\text{O}$  0.05 g

NH<sub>4</sub>Cl 0.25 g

KCl 0.36 g

HEPES 2.38 g

Yeast extract 0.5 g

Trace elements solution (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  (Mohr's salt) 784.0 mg

HCl (concentrated) 5.0 ml

CoCl<sub>2</sub>  $\times \text{H}_2\text{O}$  238.0 mg

(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub>  $\times 6 \text{ H}_2\text{O}$  395.0 mg

Na<sub>2</sub>MoO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  24.0 mg

Na<sub>2</sub>WO<sub>4</sub>  $\times 2 \text{ H}_2\text{O}$  33.0 mg

ZnSO<sub>4</sub>  $\times 7 \text{ H}_2\text{O}$  144.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{Na}_2\text{SeO}_4$  94.0 mg

$\text{H}_3\text{BO}_3$  6.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  99.0 mg

Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with water and other salts are dissolved in the sequence indicated.

*Vitamin solution according to Wolin et al.:*

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine 100.0 mg

Riboflavin 50.0 mg

Pantotenoic acid 50.0 mg

*p*-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B<sub>12</sub> 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution and trace elements from sterile stock solution.

### **386. SALINICOLA SALARIUS MEDIUM**

Tryptone 5.0 g

Yeast extract 2.5 g

D-Glucose 1.0 g

NaCl 130.0 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min.

### **387. NATRANAEROBIUS MEDIUM**

$\text{KH}_2\text{PO}_4$  0.2 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.1 g

KCl 0.2 g

$\text{NH}_4\text{Cl}$  0.5 g

NaCl 100 g

Yeast extract (Difco) 6.0 g

Tryptone (Difco) 6.0 g

Trace element solution SL-10 (see below) 1.0 ml

Vitamin solution (see below) 10 ml

$\text{Na}_2\text{CO}_3$  68.0 g

$\text{NaHCO}_3$  38.0 g

L-Cysteine-HCl  $\times \text{H}_2\text{O}$  0.7 g

Glucose 5.0 g

Distilled water to 1000.0 ml

pH 10.5 (measured at 60°C)

Dissolve ingredients (except carbonates, cysteine, vitamins and sucrose), then sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Add carbonate, bicarbonate and cysteine and adjust pH to 10.5. Dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins and glucose from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and sterilized by filtration.

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 0.1 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4.

### **388. THERMODESULFOBIUM ACIDIPHILUM MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.33 g  
Na<sub>2</sub>SO<sub>4</sub> 2.8 g  
Trace mineral solution SL-10 (see below) 1.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml  
Yeast extract 3.0 g  
Vitamin solution (see below) 10.0 ml  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.45 g  
Distilled water 1000.0 ml

Dissolve ingredients except yeast extract, vitamins and sulfide. Adjust pH to 4.5 with H<sub>2</sub>SO<sub>4</sub> and sparge medium with 100% CO<sub>2</sub> gas for 10 – 15 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes and autoclave. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The sulfide stock solution (3% w/v) should be neutralized before use. The pH of the complete medium should be at 4.5.

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 0.1 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

### **389. TRICHOCOCCUS MEDIUM**

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.12 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.19 g  
KH<sub>2</sub>PO<sub>4</sub> 0.45 g  
K<sub>2</sub>HPO<sub>4</sub> 0.45 g  
NaCl 0.9 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.9 g  
Yeast extract 2.0 g  
Trypticase peptone 2.0 g  
Na-resazurin solution (0.1% w/v) 0.5 ml  
L-Cysteine-HCl × H<sub>2</sub>O 1.0 g  
Na<sub>2</sub>CO<sub>3</sub> 2.5 g  
D-Glucose 2.0 g  
Distilled water 1000.0 ml

Dissolve ingredients (except cysteine, carbonate and glucose), adjust pH to 7.0 and sparge medium with 100% CO<sub>2</sub> gas for 10 – 15 min to make it anoxic. Add the cysteine and carbonate, then equilibrate the medium with the CO<sub>2</sub> gas to pH 7.0. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose from an anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration.

### **390. SULFURIMONAS MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2H<sub>2</sub>O 0.03 g  
KH<sub>2</sub>PO<sub>4</sub> 0.16 g  
MgCl<sub>2</sub> × 6H<sub>2</sub>O 0.33 g  
NaCl 10.0 g  
NaHCO<sub>3</sub> 2.0 g  
Elemental sulfur 5.0 g  
KNO<sub>3</sub> 1.0 g  
Trace element solution (see below) 1 ml  
Vitamin solution (see below) 1 ml  
pH of 7.5–8.0 (measured at 25 °C)  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine 0.1 mg  
Riboflavin 5.0 mg  
Pantotenoic acid 5.0 mg  
*p*-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4.

The anoxic medium is prepared by boiling and cooling it under N<sub>2</sub> flow. No reducing agents are added. Elemental sulfur as electron donor is added to the Hungate tubes prior sterilization; potassium nitrate from anoxic sterile sock solution was added as electron acceptor just before inoculation.

**391. ALKALIBACULUM MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.033 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
NaOH 10.0 g  
NaHCO<sub>3</sub> 2.0 g  
Glucose 2.0 g  
Yeast extract 50 mg  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Resazurin 0.2 mg  
Trace element solution (see below) 1 ml  
Vitamin solution (see below) 1 ml  
pH of 8.0-8.5 (measured at 25 °C)

*Trace element solution:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O (*Mohr's salt*) 784.0 mg  
HCl (concentrated) 5.0 ml  
CoCl<sub>2</sub> × H<sub>2</sub>O 238.0 mg  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 144.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg  
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

*Vitamin solution:*

Biotin 20.0 mg  
Folic acid 20.0 mg  
Pyridoxine 0.1 mg  
Riboflavin 50.0 mg  
Pantotenoic acid 50.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Thiamine-HCl 50.0 mg  
Nicotinic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
Lipoic acid 50.0 mg  
Distilled water 1000.0 ml

The medium is prepared anaerobically. NaHCO<sub>3</sub>, NaOH and vitamins, Na<sub>2</sub>S × 9 H<sub>2</sub>O are added after boiling and cooling of the medium under 100% N<sub>2</sub> in gas phase. Adjust the pH between 6.0 and 6.5 with sterile HCl or NaOH after autoclaving. Yeast extract must be added to the final solution before inoculation.

### **392. CALORIBACTERIUM MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.033 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 0.33 g  
NaOH 10.0 g  
NaHCO<sub>3</sub> 2.0 g  
Glucose 2.0 g  
Yeast extract 50 mg  
Na<sub>2</sub>S × 9 H<sub>2</sub>O 0.5 g  
Resazurin 0.2 mg  
Trace element solution (see below) 1 ml  
Vitamin solution (see below) 1 ml  
pH of 7.5–8.0 (measured at 25°C)

#### *Trace element solution:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O (*Mohr's salt*) 784.0 mg  
HCl (concentrated) 5.0 ml  
CoCl<sub>2</sub> × H<sub>2</sub>O 238.0 mg  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 395.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 33.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 144.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 2.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 94.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 99.0 mg  
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

#### *Vitamin solution:*

Biotin 20.0 mg  
Folic acid 20.0 mg  
Pyridoxine 0.1 mg  
Riboflavin 50.0 mg  
Pantotenoic acid 50.0 mg  
*p*-Aminobenzoic acid 50.0 mg  
Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
Lipoic acid 50.0 mg  
Distilled water 1000.0 ml

The medium is prepared anaerobically. NaHCO<sub>3</sub>, NaOH and vitamins, Na<sub>2</sub>S × 9H<sub>2</sub>O are added after boiling and cooling of the medium under 100% CO<sub>2</sub> in gas phase. Adjust the pH between 6.0 and 6.5 with sterile HCl or NaOH after autoclaving. Yeast extract must be added to the final solution before inoculation.

### **393. PLATE COUNT AGAR WITH 0.5% NaCl**

Tryptone 5.0 g  
Yeast extract 2.5 g  
Glucose 1.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.0±0.2  
Autoclave at 121°C for 15 min.

### **394. LICHENIBACTERIUM MINOR MEDIUM**

Glucose 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NaCl 0.2 g  
NH<sub>4</sub>NO<sub>3</sub> 0.2 g  
MgSO<sub>4</sub> × 5 H<sub>2</sub>O 0.04 g  
Yeast extract(without sodium chloride) 0.2 g  
Vitamin solution (see below) 1 ml  
Distilled water 1000.0 ml  
pH 5.0-6.5

*Vitamin solution:*

*p*-Aminobenzoate 1.0 mg  
Biotin 0.2 mg  
Nicotinic acid 2.0 mg  
Thiamine-HCl 1.0 mg  
Ca-pantothenate 0.5 mg  
Pyridoxamine 5.0 mg  
Vitamin B<sub>12</sub> 2.0 mg  
Distilled water 1000.0 ml

Prepare the medium without the vitamin solution. Autoclave at 121°C for 15 min and add the vitamin solution from a filter-sterilized stock solution.

### **395. FRIGORIGLOBUS TUNDRICOLA MEDIUM**

Glucose 0.5 g  
Yeast extract 0.25 g  
Peptone 0.25 g  
N-acetylglucosamine 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>NO<sub>3</sub> 0.1 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml (800.0 ml for solid medium)  
pH 5.8-6.0  
Autoclave at 121°C for 15 min

For solid medium 8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

### **396. LACIPIRELLULA PARVULA MEDIUM**

Glucose 0.5 g  
Yeast extract 0.25 g  
Peptone 0.25 g  
N-acetylglucosamine 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>NO<sub>3</sub> 0.1 g  
CaCl<sub>2</sub> × 6 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml (800.0 ml for solid medium)  
pH 6.8-7.5  
Autoclave at 121°C for 15 min

For solid medium 8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

### **397. JANTHINOBACTEIUM LIVIDUM MEDIUM**

Peptone 30.0 g  
Na<sub>2</sub>HPO<sub>4</sub> 2.0 g  
NaCl 3.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
Autoclave at 121°C for 15 min

### **398. RHODOPSEUDOMONAS PARAPALUSTRIS MEDIUM**

Tryptone 5.0 g  
Yeast extract 2.5 g  
Glucose 1.0 g  
Distilled water 1000.0 ml  
pH 6.8±0.2

### **399. GEMMATA PALUSTRIS MEDIUM**

*Hutner's* basal salts medium (see below) 20.0 ml  
Peptone 5.0 g  
Yeast extract 0.25 g  
Distilled water 965.0 ml (less for plates)  
Adjust pH to 6.8-7.5 with 5 M KOH  
After autoclaving and cooling (see below for plates) add to the medium  
Vitamin solution 5.0 ml  
*Hutner's basal salts medium:*  
Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.0127 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.099 g  
Metal salt solution "44" (see below) 50.0 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.  
*Metal solution "44":*



Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

*Vitamin solution:*

Biotin 4.0 mg  
Folic acid 4.0 mg  
Pyridoxine-HCl 20.0 mg  
Riboflavin 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 10.0 mg  
Nicotinamide 10.0 mg  
D-Ca-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 0.2 mg  
*p*-Aminobenzoic acid 10.0 mg  
Distilled water 1000.0 ml  
Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

#### **400. THIOTHRIX MEDIUM**

NH<sub>4</sub>Cl 0.3 g  
CaCl<sub>2</sub> 0.03 g  
KH<sub>2</sub>PO<sub>4</sub> 0.01 g  
K<sub>2</sub>HPO<sub>4</sub> 0.022 g  
Na<sub>2</sub>HPO<sub>4</sub> × 7 H<sub>2</sub>O 0.035 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.05 g  
FeCl<sub>3</sub> × 6H<sub>2</sub>O 0.002 g  
NaNO<sub>3</sub> 0.30 g  
Peptone 0.2 g  
Na-acetate (10%) 2.5 ml  
Na-lactate (10%) 2.5 ml  
NaHCO<sub>3</sub> (10%) 5.0 ml  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5 H<sub>2</sub>O (10%) 10.0 ml  
Vitamin solution (see below) 1.0 ml  
Trace element solution according to *Hogland*: (see below) 1.0 ml  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
*p*-Aminobenzoic acid 50.0 mg

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> × 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> 10.0 mg

NiCl<sub>2</sub> × 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

pH 7.5 (adjust with 1% HCl)

To prepare the trace element solution, preliminarily acidify water to pH 3.0-4.0 with HCl. Sterilize thiosulfate, acetate, lactate, bicarbonate, trace elements and vitamins separately and add to the main medium prior to inoculation. Sterilize the vitamin solution by filtration, others solutions and base medium by autoclaving at 121°C for 15 min.

#### **401. LICHENICOCCUS ROSEUS MEDIUM (LRM)**

Glucose 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NaCl 0.2 g

NH<sub>4</sub>NO<sub>3</sub> 0.2 g

MgSO<sub>4</sub> × 5 H<sub>2</sub>O 0.04 g

Yeast extract (without sodium chloride) 0.2 g

Vitamin solution (see below) 1 ml

Distilled water 1000.0 ml

pH 4.5

*Vitamin solution:*

*p*-Aminobenzoic acid 1.0 mg

Biotin 0.2 mg

Nicotinic acid 2.0 mg

Thiamine-HCl 1.0 mg

D-Ca-pantothenate 0.5 mg

Pyridoxamine 5.0 mg

Vitamin B<sub>12</sub> 2.0 mg

Distilled water 100.0 ml

Prepare the medium without the vitamin solution. Autoclave at 121°C for 15 min and add the vitamin solution from a filter-sterilized stock solution.

#### **402. PROSTHECODIMORPA STALEYI MEDIUM (PSM)**

Glucose 0.3 g

Na<sub>2</sub>HPO<sub>4</sub> 0.071 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.25 g

Yeast extract 0.05 g

Peptone 0.1 g

Vitamin solution (see below) 10.0 ml

Hutner's salt solution (see below) 20.0 ml

Distilled water 970.0 ml

*Vitamin solution :*

Biotin 2.0 mg

Folic acid 2.0 mg

Thiamine-HCl 5.0 mg

D-Ca- Panthothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 мг  
Riboflavin 5.0 мг  
Distilled water 1000.0 мл  
*Hutner's Salt Solution:*  
Nitrilotriacetic acid 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.335 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 99.0 mg  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> × 4 H<sub>2</sub>O 9.25 mg  
"Metals 44" 50.0 ml  
Distilled water 1000.0 мл  
*"Metals 44":*  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1.095 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g  
EDTA-Na 0.25 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.154 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.2 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 100.0 мл

#### **403. ALKALISPIRILLUM MOBILE MEDIUM (AMM)**

NaCl 20.0 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.8 g  
Na-acetate 2.0 g  
Yeast extract 1.0 g  
Trace elements (see below) 1.0 ml  
MgCl<sub>2</sub> × 7 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g  
NH<sub>4</sub>Cl 0.8 g  
Na<sub>2</sub>CO<sub>3</sub> 21.2 g  
NaHCO<sub>3</sub> (8 %) 168.0 ml  
Distilled water 832.0 ml  
*Trace element solution (A):*  
FeCl<sub>2</sub> × 4 H<sub>2</sub>O 1.8 g  
CoCl<sub>2</sub> × 6 H<sub>2</sub>O 250.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 10.0 mg  
CuCl<sub>2</sub> × 2 H<sub>2</sub>O 10.0 mg  
MnCl<sub>2</sub> × 4 H<sub>2</sub>O 70.0 mg  
ZnCl<sub>2</sub> 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 500.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 30.0 mg  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 10.0 mg  
Distilled water 1000.0 ml  
For dissolving adjust pH to about 3 with 1 N HCl.

We recommend that liquid and solid media be made by autoclaving the Na<sub>2</sub>CO<sub>3</sub> separately, otherwise the high pH will cause ammonia to be lost. When making media solidified with agar (20 g/l) the agar should also not be autoclaved with the Na<sub>2</sub>CO<sub>3</sub> otherwise the agar will be hydrolysed. When mixing the ingredients for media cool all components to about 55 °C otherwise hydrolysis of the agar may occur. Adjust pH after autoclaving by adding the NaHCO<sub>3</sub> (8 %) to pH 9.0. Please note that the NaHCO<sub>3</sub> solution should be filter sterilized

**404. PEPTONE MEAT AGAR WITH MANGANESE (PMA-Mn)**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 100 mg  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min.

**405. MAGNETOSPIRILLUM KUZNETSOVII MEDIUM (MKM)**

KH<sub>2</sub>PO<sub>4</sub> 0.68 g  
NaNO<sub>3</sub> 0.12 g  
L(+)-Tartaric acid 0.37 g  
Succinic acid 0.37 g  
Na-acetate × 3 H<sub>2</sub>O 0.05 g  
Vitamin solution (see below) 10.0 ml  
Trace element solution (see below) 5.0 ml  
Fe(III) quinate solution (see below) 2.0 ml  
Na-resazurin solution (0.1% w/v) 0.5 ml

Na-thioglycolate 0.05 g  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 1.5 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 3.0 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 0.5 g  
NaCl 1.0 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.1 g  
CoSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 0.18 g  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 0.01 g  
KAl(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 0.02 g  
H<sub>3</sub>BO<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>SeO<sub>3</sub> × 5 H<sub>2</sub>O 0.3 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 0.4 mg  
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
D-Ca-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
*p*-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

*Ferric Quinate Solution, 0.01 M:*

FeCl<sub>3</sub> × 6 H<sub>2</sub>O 4.5 g

Quinic acid 1.9 g

Distilled water 1000.0 ml

Sterilize by filtration under 100% N<sub>2</sub> gas atmosphere.

Dissolve ingredients (except thioglycolate) and adjust pH to 6.75 with NaOH. Sparge medium with 100% N<sub>2</sub> gas for 30-45 min and dispense under the same gas atmosphere into anoxic Hungate – type tubes to 50% of their volume. Before inoculation add thioglycolate from a 0.5% (w/v) stock solution, freshly prepared under 100% N<sub>2</sub> gas and filter-sterilized. Then add sterile air to a concentration of 1.0 % O<sub>2</sub> in the vial.

#### **406. COLUMBIA AGAR WITH BLOOD (CA-B)**

Special mixture of peptones 23.0 g

Defibrinated blood 50.0 g

Starch 1.0 g

NaCl 5.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.3±0.2.

Adjust pH to 7.3±0.2. Autoclave at 121°C for 15 min.

#### **407. PSEUDOALTEROMONAS TELLURITIREDUCTENS MEDIUM (PTM)**

MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.3 g

NH<sub>4</sub>Cl 0.3 g

CaCl<sub>2</sub> × 2 H<sub>2</sub>O 0.05 g

Na-acetate × 3 H<sub>2</sub>O 10.0 g

Yeast extract 0.5 g

Casamino acids 0.5 g

NaCl 15.0 g

Trace elements solution (see below) 2.0 ml

Vitamin solution (see below) 2.0 ml

Distilled water 1000.0 ml

*Trace elements solution:*

MnCl<sub>2</sub> × 4 H<sub>2</sub>O 0.3 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.03 g

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 80 mg

Thiamine-HCl 400.0 mg

Nicotinic acid 400.0 mg

Vitamin B<sub>12</sub> 20.0 µg

Distilled water 1000.0 ml

After autoclaving at 121°C for 15 min adjust to pH 7.8 with NaHCO<sub>3</sub>. Add the vitamin solution and trace elements solution from sterile stock solutions.

#### **408. PLANCTOMYCETES SP. MEDIUM (PspM)**

*Hutner's* basal salts medium (see below) 20.0 ml

Peptone Bacto 1.0 g

Yeast extract Bacto 1.0 g

HEPES 2.38 g

Artificial seawater (see below) 250.0 ml

Distilled water 690.0 ml (less for plates)

Adjust pH to 7.5 with 5 M KOH

After autoclaving and cooling (please see below for plates) add to the medium:

Vitamin solution 5.0 ml  
N-Acetylglucosamine 20.0 ml of 50.0 g/l stock solution  
Trace element solution 1.0 ml

*Artificial sea water:*

NaCl 46.94 g  
Na<sub>2</sub>SO<sub>4</sub> 7.84 g  
MgCl<sub>2</sub> × 6 H<sub>2</sub>O 21.28 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 2.86 g  
NaHCO<sub>3</sub> 0.384 g  
KCl 1.384 g  
KBr 0.192 g  
H<sub>3</sub>BO<sub>3</sub> 0.052 g  
SrCl<sub>2</sub> × 6 H<sub>2</sub>O 0.08 g  
NaF 0.06 g  
Distilled water 1000.0 ml

Do not autoclave. Due to high salt concentration, no filtration. Sometimes precipitation after a while or fungal growth observed, thus better prepare fresh. Store at room temperature.

*Hutner's basal salts medium:*

Nitrilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> × 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> × 2 H<sub>2</sub>O 0.01267 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.099 g  
Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).

Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

*Metal solution "44":*

Na-EDTA 250.0 mg  
ZnSO<sub>4</sub> × 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> × 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> × 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

*Vitamin solution:*

Biotin 4.0 mg  
Folic acid 4.0 mg  
Pyridoxine-HCl 20.0 mg  
Riboflavine 10.0 mg  
Thiamine-HCl × 2 H<sub>2</sub>O 10.0 mg  
Nicotinamide 10.0 mg  
D-Ca-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 0.2 mg  
*p*-Aminobenzoic acid 10.0 mg  
Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

*Trace element solution:*

Na-Nitrilotriacetat 1.5 g  
MnSO<sub>4</sub> × H<sub>2</sub>O 500.0 mg

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 100.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> × 6 H<sub>2</sub>O 100.0 mg  
ZnCl<sub>2</sub> 100.0 mg  
NiCl<sub>2</sub> × 6 H<sub>2</sub>O 50.0 mg  
H<sub>2</sub>SeO<sub>3</sub> 50 mg  
CuSO<sub>4</sub> × 5 H<sub>2</sub>O 10.0 mg  
AlK(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O 10.0 mg  
H<sub>3</sub>BO<sub>3</sub> 10.0 mg  
NaMoO<sub>4</sub> × 2 H<sub>2</sub>O 10.0 mg  
Na<sub>2</sub>WO<sub>4</sub> × 2 H<sub>2</sub>O 10.0 mg  
Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

15.0 g/l Agar (Bacto) 3x washed with distilled water, autoclave separately and take volume of distilled water into account.

or

8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

#### **409. SEA WATER MEDIUM FOR *HALOMONAS* (SWM-H)**

Sea salt 37.9 g  
Yeast extract 3.0 g  
Peptone 10.0 g  
NaCl 60 g  
Agar 20.0 g  
Distilled water to 1000.0 ml  
pH 7.2-7.4  
Autoclave at 121°C for 15 min.

#### **410. *SHINELLA* SP. MEDIUM (SspM)**

Tryptone 5.0 g  
Yeast extract 2.5 g  
Glucose 1.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.0±0.2  
Autoclave at 121°C for 15 min.

#### **411. MEDIUM FOR *METHYLOLIGELLA HALOTOLERANS* (MMH)**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g  
Methanol 20.0 ml  
Distilled water 1000.0 ml  
pH 7.0  
Autoclave at 121°C for 15 min. Methanol is filter sterilized.

#### **412. MEDIUM FOR *PARACOCCLUS SIMPLEX* (MPS)**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> × 7 H<sub>2</sub>O 0.025 g

NaCl 0.5 g

FeSO<sub>4</sub> × 7 H<sub>2</sub>O 0.02 g

Methylamine solution 30.0 ml

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min. 10 % solution of methylamine autoclave at 111°C for 30 min.