4.0 MEDIA FORMULATIONS

NCIM catalogue and packing slips specify the medium recommended by NCIM as optimum for initial revival or subculture of each strain.
When components are critical or specified in the original formulation received from a depositor, the commercial brand of an ingredient is given.

Unless noted otherwise, media are sterilized by autoclaving at 121°C for 20 min.

1. Acetobacter aceti

Tryptone	1.0	g
Yeast extract	1.0	g
Agar	2.0	g
Distilled water	100	ml

Adjust pH to 6.0. Steam the medium to melt agar and add the following components.

Glucose	1.0	g
$CaCO_3$	1.0	g

Dispense the medium into tubes while stirring constantly to ensure equal distribution of $CaCO_3$.
Sterilze at 110℃ for 20 min.

2. Acetobacter suboxydans

Sorbitol	5.0	g
Yeast extract	0.5	g
Distilled water	100	ml
Agar	2.0	g

Adjust pH to 6.2

3. A.V. Medium (Streptosporangium)

L-arginine	0.03	g
Glucose	0.1	g
Glycerol	0.1	g
K_2HPO_4	0.03	g
$MgSO_4.7H_2O$	0.03	g
NaCl	0.03	g
Yeast extract	0.03	g
Agar	2.0	g
Distilled water	100.0	ml

4. Barr's Medium (Sulphur bacteria)

K_2HPO_4	0.05	g
NH_4Cl	0.1	g
$CaSO_4$	0.2	g
$MgSO_4.7H_2O$	0.2	g
Sodium lactate	0.7	g
$Fe(NH_4)_2(SO_4)_2$	0.05	g
Distilled water	100.0	ml

The medium is sterilized for three consecutive days at 121°C for 20 min and the final pH is adjusted to 7.0 - 7.5.

*After first sterilization the pH of the medium is adusted to 8.0 and after second sterilization the pH is adjusted to 7.5. Before the inoculation, the medium is boiled for 15-20 min in boiling water bath and cooled.

5. Burk's medium (Azotobacter)

K_2HPO_4	0.08	g
KH_2PO_4	0.02	g
$MgSO_4.7H_2O$	0.02	g
NaCl	0.02	g
$CaSO_4$	0.01	g
*Fe-Mo mixture	0.1	ml
Sucrose	2.0	g
H_3BO_3	10.0	µg
$ZnSO_4.7H_2O$	10.0	µg
$MnSO_4.4H_2O$	1.0	µg
$CuSO_4.5H_2O$	0.30	µg
KI	0.10	µg
Distilled water	100	ml
Agar	2.0	g
Adjust pH to 7.3		

Fe - Mo mixture

$FeCl_3.6H_2O$	1.45	g
$Na_2MoO_4.2H_2O$	0.253	g
Distilled water	100	ml

6. CH Medium (Halobacterium)

Casamino acids	0.75	g
Yeast extract	1.0	g
Tri-sodium citrate	0.3	g
KCl	0.2	g
$MgSO_4.7H_2O$	2.0	g
$FeSO_4.7H_2O$	5.0	mg
$MnCl_2.H_2O$	40.0	µg
NaCl	20.0	g
Agar(Difco)	2.2	g
Distilled water	100	ml

Before adding the agar filter the media, adjust the pH to 7.0

7. Chlorella Medium

To Fog's medium add 0.2% KNO_3

8. Conrad's Medium (Xylose medium)

KH_2PO_4	0.4	g
Na_2HPO_4	0.05	g
NH_4Cl	0.3	g
NaCl	0.05	g
$MgSO_4.7H_2O$	0.04	g
$CaCl_2.2H_2O$	1.0	mg
$ZnSO_4.7H_2O$	10.0	µg

Suspend in 70 ml distilled water and adjust pH to 7.0.
Add 2.0 g of xylose in 30 ml. Autoclave separately and mix aseptically.

9. Cooked Meat Medium (Clostridium)

Beef extract	4.5	g
Dextrose	0.2	g
Proteose peptone	2.0	g
NaCl	0.5	g
Distilled water	100	ml

Adjust pH to 7.2.

10. Cooked Meat Medium (Hi-Media M149)

11. Cytophaga Medium

KH_2PO_4	0.1	g
$(NH_4)_2SO_4$	0.1	g
$MgSO_4.7H_2O$	0.02	g
$CaCl_2.2H_2O$	0.01	g
$FeCl_3.6H_2O$	2.0	mg
Yeast extract	0.01	g
Glucose	0.1	g
Distilled water	100	ml
Agar	2.0	g

Adjust pH to 7.0

12. Czapeck Dox Medium (Aspergillus candidus)

A. Glucose	20.0	g
Distilled water	150	ml
B. KH_2PO_4	1.0	g
Distilled water	100	ml
C. KCl	0.5	g
$MgSO_4.7H_2O$	0.5	g
$FeSO_4.7H_2O$	50.0	mg
$ZnSO_4.7H_2O$	50.0	mg
$NaNO_3$	1.0	g
Distilled water	150	ml
D. Agar	20.0	g
Distilled water	600	ml

Sterilize solutions A,B,C and D separately and mix the solutions aseptically.

13. Dubos Medium (Cellulomonas)

$NaNO_3$	0.5	g
K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	0.5	g
KCl	0.5	g
$FeSO_4.7H_2O$	10.0	mg
Yeast extract	0.5	g
Cellulose CP 123	5.0	g
Distilled water	1.0	L

Adjust pH to 6.5

14. Enrichment Medium (Lactic cultures)

Glucose	5.0	g
Lactose	5.0	g
Liver extract	10.0	g
Sodium acetate	6.0	g
Yeast extract	5.0	g
*Salt A solution	5.0	ml
*Salt B solution	5.0	ml
Distilled water	1.0	L
Agar	20.0	g

Adjust pH to 7.6

Salt A solution

KH_2PO_4	10.0	g
K_2HPO_4	10.0	g
Distilled water	100	ml

Salt B solution

$MgSO_4.7H_2O$	4.0	g
NaCl	0.2	g
$MnSO_4.4H_2O$	0.2	g
$FeSO_4.7H_2O$	0.2	g
Distilled water	100	ml

15. Euglena Medium

Dextrose	1.0	g
Tryptone	1.0	g
Liver extract	1.0	g
Vitamin B_{12}	20.0	mcg
Distilled water	1.0	L
Agar(Difco)	15.0	g

Adjust pH to 3.5-3.6

Sterilization at 110°C for 10 min.

For solid medium autoclave agar separately and mix the medium aseptically.

16. Flavobacterium dehydrogenans Medium

Peptone	5.0	g
Beef extract	3.0	g
K_2HPO_4	1.5	g
KH_2PO_4	1.5	g
Distilled water	1.0	L
Agar(Difco)	15.0	g

Adjust pH to 7.0

17. Fog's Medium (for Algae)

$MgSO_4.7H_2O$	0.2	g
K_2HPO_4	0.2	g
*Micronutrient solution	1.0	ml
$CaCl_2.H_2O$	0.1	g
*Fe-EDTA solution	5.0	ml
Distilled water	1.0	L
Agar(Difco)	12.0	g

Adjust pH to 7.5

***Micronutrient solution**

H_3BO_3	286.0	mg
$MnCl_2.4H_2O$	181.0	mg
$ZnSO_4.7H_2O$	22.0	mg
$Na_2MoO_4.2H_2O$	39.0	mg
$CuSO_4.5H_2O$	8.0	mg
Distilled water	100.0	ml

***Fe-EDTA solution**

In hot water dissolve 745.0 mg of Na_2EDTA and then add 557.0 mg of $FeSO_4.7H_2O$. Boil the solution for few minutes and make the volume to 100.0 ml.

18. Frize Medium (Neurospora crassa)

Yeast extract	0.8	g
Malt extract	1.7	g
Sucrose	1.7	g
Glucose	1.7	g
*Vitamin solution	3.3	ml
Hydrolyzed casein	1.7	ml
Distilled water	1.0	L
Adjust the pH to 5.5-6.0		
Agar	2.0	g

***Vitamin solution**

Thiamine	100.0	mg
Riboflavin	50.0	mg
Pyridoxin	50.0	mg
Pantothenic acid	200.0	mg
p-Aminobenzoic acid	50.0	mg
Nicotinamide	200.0	mg
Choline	200.0	mg
Inositol	400.0	mg
Folic acid	4.0	mcg
*Alkali hydrolyzed yeast extract	0.5	g
Distilled water	1.0	L

***Alkali hydrolyzed yeast extract**

20 g of yeast extract (Difco) is dissolved in 200.0 ml of 0.5N NaOH and autoclaved at 121°C for 30 min, neutralize with glacial acetic acid, autoclave again at 121°C for 10 min to coagulate protein and filter. The pH of the solution is adjusted to 1.5 with HCl. Shake with 20.0 g of activated charcoal for 20 min. Filter and neutralize with NaOH and store in refrigerator.

18 a. Holten Medium for yeast

Peptone	5.0	g
Tryptone	0.15	g
Desicated oxbile	1.5	g
Fructose	5.0	g
Sucrose	5.0	g
Mannitol	5.0	g
Phenyl alanine	1.0	g
Vancomycin	10.0	mg
Ampicillin	10.0	mg
Bavistin	1.0	µg
Phenol Red indicator	0.02	ml
Distilled water	1.0	L
Adjust pH to 6.8		
Agar	20.0	g

19. Jaggery medium (Osmophilic yeast)

Jaggery	60.0	g
Yeast extract	0.1	g
KH_2PO_4	50.0	mg
$MgSO_4.7H_2O$	50.0	mg
NaCl	10.0	mg
$CaCl_2$	10.0	mg
Distilled water	100.0	ml
Agar	2.0	g
Adjust pH to 6.5-7.0		

20. Klebsiella Medium

Bacto Tryptone	10.0	g
Yeast extract	1.0	g
Glucose	1.0	g
Nacl	8.0	g
Distilled water	1.0	L
Agar (Difco)	15.0	g
Adjust pH to 7.0		

21. 9K Medium (Thiobacillus ferrooxidans)

Solution A

$(NH_4)_2SO_4$	3.0	g
KCl	0.1	g
K_2HPO_4	0.5	g
$MgSO_4.7H_2O$	0.5	g
$Ca(NO_3)_2$	0.01	g
Distilled water	700.0	ml

Solution B

$FeSO_4.7H_2O$	44.22	g
H_2SO_4 (1N)	10.0	ml
Distilled water	290.0	ml

Solution A & B are sterilized separately and then mixed asceptically.

21a. 9K+ Glucose (Thiobacillus acidophilus)

Glucose	10	g
$(NH_4)_2SO_4$	3.0	g
KH_2PO_4	0.5	g
$MgSO_4.7H_2O$	0.5	g
KCl	0.1	g
$Ca(NO_3)_2$	0.01	g
$FeSO_4.7H_2O$	0.01	g
Distilled water	1.0	L

For liquid medium , the pH should be adjusted to 3.5 with H_2SO_4. The glucose solution and basal 9K solution must be sterilized separately and added aseptically.

22. Lactobacillus leichmanni medium

Dextrose	1.0	g
Peptone	0.75	g
Yeast extract	0.75	g
KH_2PO_4	0.2	g
Tomato juice	10.0	ml
Tween 80	1.0	ml
Distilled water	89.0	ml
Agar (if needed)	2.0	g

Adjust pH to 6.8 - 7.0

*Tomato juice

Boil 2-3 tomatoes for 10 min. Remove the skin and squeez the tomatoes. Filter the juice, adjust the pH to 7.0, centrifuge and store the clear filtrate at -20°C.

23. Leuconostoc oenos medium

Glucose	1.0	g
Peptone	1.0	g
Yeast extract	0.5	g
$MgSO_4$. $7H_2O$	20.0	mg
$MnSO_4.H_2O$	5.0	mg
*Tomato juice	25.0	ml
Distilled water	75.0	ml
Agar (if needed)	2.0	g

Adjust pH to 4.8

Sterilize at 121°C for 15 min

*Refer L.lechmannii medium (22)

24. Link Medium for Aspergillus candidus

Glycerine	1.0	g
Yeast extract	0.5	g
Glucose	1.0	g
NaCl	1.0	g
KH_2PO_4	10.0	mg
$MgSO_4.7H_2O$	5.0	mg
Distilled water	100.0	ml
Agar	2.0	g

25. Lochhead's Medium (Rhizobium cultures)

KH_2PO_4	0.4	g
$MgSO_4.7H_2O$	80.0	mg
NaCl	80.0	mg
$CaSO_4$	50.0	mg
$CaCO_3$	2.0	g
Sucrose	10.0	g
Mannitol	4.0	g
Maltose	2.0	g
Yeast extract	1.0	g
Malt extract	0.24	g
*Soil extract	200.0	ml
Distilled water	800.0	ml
Agar	22.0	g

*Soil extract

To 200 g of garden soil add 1.0 g of $NaHCO_3$ and 1000 ml of water. Autoclave at 121°C for 60 min. Allow the mixture to settle and decant it. If necessary centrifuge and store at -20°C.

25a. Lowenstein Jensen Medium (Mycobacterium smegmatis)

KH_2PO_4	2.50	g
$MgSO_4$. $7H_2O$	0.24	g
Mg citrate	0.60	g
L-Asparagine	3.60	g
Potato flour	34.0	g
Malachite green	0.40	g

Suspend 37.3 g in 600 ml water containing 12.5 ml glycerol. Autoclave at 15 lbs 15 min. Meanwhile prepare 1000ml whole egg emulsion collected aseptically. Admix egg and base gently to obtain uniform mixture. Distribute in sterile screw capped tubes. Arrange in slanted position and coagulate the medium in an inspissator water bath or autoclave at 85-90°C for 45 min.

26. LB Medium (Luria broth) / Agar

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	10.0	g
Distilled water	1.0	L
Agar (if needed)	20.0	g

Adjust pH to 7.0

26 a. LB Medium (Luria broth) / Agar with 1%CMC

Prepare LB Medium (No. 26). Adjust the pH to 9.0 with 10% Na_2CO_3.

27. M2 Medium (Neurospora crassa)

Glucose	1.0	g
Glycerine	1.0	g
Yeast extract	0.5	g
KH_2PO_4	30.0	mg
$MgSO_4.7H_2O$	10.0	mg
Distilled water	100.0	ml
Agar	2.0	g

Adjust pH to 6.8 to 7.0

28. Microcyclus Medium

Beef extract	0.3	g
Tryptone	0.5	g
Dextrose	0.1	g
Yeast extract	0.1	g
Distilled water	100.0	ml
Agar	2.0	g

29. MGYP medium (Yeast culture)

Malt extract	0.3	g
Glucose	1.0	g
Yeast extract	0.3	g
Peptone	0.5	g
Distilled water	100.0	ml
Agar	2.0	g

Adjust pH to 6.4-6.8

30. MGYP+ Thiostrepton (25 µg/ml)

31. Milk Medium for lactic cultures

Skim milk powder	10.0	g
Yeast extract	0.5	g
$CaCO_3$	1.0	g

Preparation of medium

Add skim milk powder to approximately 20-30 ml of distilled water, make smooth paste and adjust the volume to 100 ml. Add yeast extract and adjust the pH to 7.3. After adding $CaCO_3$ mix thoroughly and distribute it in test tubes. Sterilize the medium 110°C for 10 min for three consecutive days.

32. MRS Medium (Lactic cultures)

Proteose peptone	10.0	g
Yeast extract	5.0	g
Beef extract	10.0	g
Dextrose	20.0	g
Tween 80	1.0	g
Ammonium citrate	2.0	g
Sodium acetate	5.0	g
$MgSO_4.7H_2O$	0.1	g
$MnSO_4.4H_2O$	0.05	g
K_2HPO_4	2.0	g
Distilled water	1.0	L

The medium is available with Hi-Media (M-369).

32a. Mycobacterium Medium

MGYP medium with 10.0% glucose

33. Mycobacterium phlei Medium

KH_2PO_4	0.5	g
$MgSO_4.7H_2O$	60.0	mg
Sodium citrate	0.25	g
Glycerine	2.0	ml
Aspargine	0.5	g
Distilled water	100.0	ml
Agar	2.0	g

Adjust pH to 7.8 with 4N KOH.
Sterilization at 110°C for 20 min.

34. MYG medium (Aspergillus mutants)

Malt extract	0.5	g
Yeast extract	0.25	g
Glucose	1.0	g
Distilled water	100.0	ml
Agar	2.0	g

35. NCIB Medium (Thiobacillus ferrooxidans)

$(NH_4)_2SO_4$	15.0	mg
KCl	5.0	mg
K_2HPO_4	5.0	mg
$MgSO_4.7H_2O$	50.0	mg
$Ca(NO_3)_2$	1.0	mg
Distilled water	100.0	ml

Prepare 10% of $FeSO_4.7H_2O$ solution and sterilize both the solutions at 121°C for 20 min separately. Add 1.0 ml of $FeSO_4$ solution per 100 ml of above salt solution.

36. NEERI Medium

Yeast extract	1.5	g
Beef extract	1.5	g
Peptone (Difco)	5.0	g
Glucose	1.0	g
*Phenol solution	5.0	ml
Distilled water	1.0	L
Agar	20.0	g

Adjust pH to 7.4
Sterilize at 115°C for 15 min.
*Phenol solution
Stock solution is prepared by adding 1.0 g of phenol to 100 ml of water.

37. Neurospora mutant Medium

Maltose	1.26	g
Yeast extract	0.08	g
Peptone	0.2	g
Malt extract	0.06	g
Distilled water	100.0	ml
Agar	2.0	g

Adjust the pH to 6.0 to 6.2.

38. Neurospora minimal Medium

Glucose	2.0	g
Ammonium nitrate	0.2	g
Ammonium tartarate	0.1	g
KH_2PO_4	0.3	g
$MgSO_4.7H_2O$	50.0	mg
NaCl	10.0	mg
$CaCl_2.2H_2O$	10.0	mg
Distilled water	100.0	ml
$ZnSO_4.7H_2O$	20.0	µg
$MnSO_4.H_2O$	20.0	µg
$CuSO_4.5H_2O$	8.0	µg
$FeSO_4.7H_2O$	2.0	µg
$Na_2MoO_4.2H_2O$	2.0	µg
Biotin	0.5	µg

Adjust pH to 4.8 - 5.0

39. Nitrobacter medium (modified Stanier's medium)

Solution I.

$MgSO_4.7H_2O$	0.2	g
K_2HPO_4	1.0	g
$FeSO_4.7H_2O$	50.0	mg
$CaCl_2.2H_2O$	20.0	mg
$MnCl_2.4H_2O$	2.0	mg
$Na_2MoO_4.2H_2O$	1.0	mg
Distilled water	1.0	L

Adjust pH to 8.5 with NaOH

Sterilization at 121°C for 15 min.

Solution II.

$NaNO_2$	6.0	g
Distilled water	100.0	ml

Solution is filter sterilized.

To 100.0 ml of solution I, 5.0 ml of solution II is added aseptically (300 mg final concentration).

40. Nitrosomonas medium (Stanier's medium)

Solution I.

K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	0.2	g
$CaCl_2.2H_2O$	20.0	mg
$FeSO_4.7H_2O$	50.0	mg
$MnCl_2.4H_2O$	2.0	mg
$Na_2MoO_4.2H_2O$	1.0	mg
Distilled water	1.0	L

Adjust pH to 8.5 with 5N NaOH. Add 0.5 % $CaCO_3$ to the medium after adjusting the pH.

Solution II.

NH_4Cl	3.0	g
Distilled water	100.0	ml

Solution is filter sterilized.

To 100.0 ml of solution I, 5.0 ml solution II is added aseptically. (150 mg final concentration)

41. Nutrient Agar

Beef extract	10.0	g
NaCl	5.0	g
Peptone	10.0	g
Distilled water	1.0	L
Agar	20.0	g

Adjust pH to 7.0-7.5

41a. Nutrient agar with pH 9.5

41b. 1/100th Nutrient agar

42. Nutrient Agar (1/2 strength) with filter paper strip.

43. Nutrient Agar with 2.0 % glucose

43a. Nutrient Agar with 2.0% NaCl

43b. Nutrient Agar with 0.5 % Glucose and 1.0% Glycerol

43c. Nutrient Agar with 0.5% Glucose

43d. Pikovskaya's Agar for phosphate solubilizing bacteria

Hi-Media product No. M520

44. Potato Dextrose Agar (Fungal cultures)

Two hundred g of peeled potatoes are cut into small pieces and suspended in 1000 ml of distilled water and steamed for 30 min. Decant the extract or filter through maslein cloth and make the final volume to 1000 ml. Add 20 g of Dextrose, 0.1 g of yeast extract and 20.0 g of agar.

45. Potato Dextrose Agar with 0.5 g / 100ml Yeast Extract & 0.5 g / 100ml Soya Peptone, pH 6.8.

46. Spirulina medium

$NaHCO_3$	10.0	g
$NaNO_3$	2.5	g
NaCl	1.0	g
K_2HPO_4	0.5	g
K_2SO_4	1.0	g
$MgSO_4.7H_2O$	0.2	g
$CaCl_2$	0.04	g
$FeSO_4.7H_2O$	0.01	g
Distilled water	1.0	L
Agar (Difco)	20.0	g
pH	8.0	

Autoclave $NaHCO_3$ and other salts separately at 15 lbs for 20 min and then mix.

46a. Spirulina medium

$NaHCO_3$	8.0	g
NaCl	5.0	g
Urea	2.2	g
K_2HPO_4	0.5	g
Phosphoric acid	0.052	ml
$FeSO_4.7H_2O$	0.05	g
Distilled water	1.0	L
Agar (Difco)	20.0	g
pH	8.0	

47. Starch Casein Agar Medium

Hi –Media M-801 (in 3.0% NaCl or artificial sea water)

48. YEB medium for Agrobacterium

Beef extract	0.5	g
Yeast extract	0.1	g
Peptone	0.5	g
Sucrose	0.5	g
$MgSO_4.7H_2O$	30.0	mg
Distilled water	100.0	ml
Agar	2.0	g

48a. YK Medium

Asparagin	5.0	g
KH_2PO_4	5.0	g
Na-citrate	2.5	g
$MgSO_4.7H_2O$	0.5	g
Fe-ammo. citrate	50.0	mg
Tween-80	0.8	ml
Distilled water	1.0	L

Adjust pH to 7.2-7.4

Add 32.0 g glycerol before autoclaving.

49. YPD medium for Zymomonas

Yeast extract	0.5	g
Peptone	0.5	g
Dextrose	2.0	g
Distilled water	100.0	ml
Agar	2.0	g

Adjust the pH to 6.2-6.5

49a. YG Salt Agar

Yeast extract	10.0	g
Agar	15.0	g
Distilled water	0.9	g

Autoclave at 121^0C/15 min and add sterilized 100 ml of 20% (w/v) glucose solution and 10.0 ml of following stock solutions.

Glucose	20.0	g/100ml
$MgCl_2$	20.0	g/100ml
$(NH_4)_2SO_4$	10.0	g/100ml
KH_2PO_4	10.0	g/100ml

50. YPSS medium (Basidiobolus microsporus)

(Yeast phosphate soluble starch agar)

Yeast extract	0.4	g
Soluble starch	1.5	g
K_2HPO_4	0.1	g
$MgSO_4.7H_2O$	0.1	g
Distilled water	100.0	ml
Agar	2.0	g

51. PPYG medium

Peptone	5.0	g
Yeast extract	1.5	g
$MgCl_2$	0.5	g
Na_2HPO_4	1.5	g
NaCl	1.5	g
Glucose	10.0	g
Na_2CO_3	10.0	g
Distilled water	1.0	L
pH	10.5	

Glucose and Na_2CO_3 should be sterilized separately and added separately

52. PPYG medium with 10% NaCl

Peptone	5.0	g
Yeast extract	1.5	g
$MgCl_2$	0.5	g
Na_2HPO_4	1.5	g
NaCl	1.5	g
Glucose	10.0	g
Na_2CO_3	10.0	g
NaCl	100.0	g
Distilled water	1.0	L
pH	10.5	

53. NCIMB medium no 206

Glucose	10.0	g
$(NH_4)_2SO_4$	3.0	g
KH_2PO_4	0.5	g
$MgSO_4.7H_2O$	1.0	g
$FeSO_4.7H_2O$	0.01	mg
KCl	0.1	g
$Ca(NO_3)_2.4H_2O$	18.0	mg
Agar	15.0	g
Distilled water	1.0	L

The pH of the liquid medium should be adjusted to 3.5 with H_2SO_4 and the pH of the solid medium should be adjusted to 4.5 with H_2SO_4 after sterilization. Sterilize the glucose solution and basal solution separately

54. NCIMB medium no. 18

Solution I

($(NH_4)_2SO_4$	0.5	g
K_2HPO_4	0.5	g
$MgSO_4.\ 7H_2O$	0.5	g
1N H_2SO_4	5.0	ml
Distilled water	1.0	L

Solution II

$FeSO_4.\ 7H_2O$	167.0	g
1N H_2SO_4	50	ml
Distilled water	100	ml

Autoclave solution I at 121^0C for 15 min and sterilize solution II by ultra-filtration. After sterilization 4 parts of solution I are added to 1 part of solution II. pH of the medium should be 3.0. **The cultures should be incubated at 30^0C under stationary condition.**

55. Gluconobacter diazotrophicus medium (NCIMB medium no. 294 or 354)

Glucose	50.0	g
Yeast extract	10.0	g
$CaCO_3$	30.0	g
Agar	25.0	g
Distilled water	1.0	L
pH	5.5	

Mix $CaCO_3$ thoroughly and cool rapidly.

56. ATCC medium no. 14

K_2HPO_4	0.08	g
KH_2PO_4	0.02	g
$MgSO_4.\ 7H_2O$	0.02	g
$CaSO_4.2H_2O$	0.01	g
$FeCl_3$	Trace	
Na_2MoO_4	Trace	
Yeast extract	0.05	g
Sucrose	2.0	g
Distilled water	100	ml
pH	7.3	
Agar (if needed)	2.0	g

57. Zobell marine agar 2216 (Hi-Media M384)

58. Tryptic Soy broth (TSA) (Difco 0730-01)

59. Todd Hewitt broth (THB) (Sigma T1438)

60. Brain heart infusion broth (BHI) (Fluka 53286)

61. MTCC medium 71

Yeast extract	2.0	g
Peptone	5.0	g
Agar	15.0	g
Soil extract	50	ml
Distilled water	950	ml

62. MTCC medium 254

Peptone	5.0	g
Yeast extract	3.0	g
Glucose	5.0	g
NaCl	5.0	g
$MgSO_4.7H_2O$	5.0	g
Oilve oil	10	ml
Distilled water	1.0	L

63. Hesterin and Schramme medium

Glucose	20.0	g
Peptone	5.0	g
Yeast extract	5.0	g
Citric acid	1.15	g
Na_2HPO_4	2.7	g
Distilled water	1.0	L
pH	5.5	

64. Thalassospira medium

Bactopeptone	5.0	g
Yeast extract	1.0	g
$FeCl_3$	0.1	g
$MgCl_2$	5.0	g
NaCl	12.0	g
$CaCl_2$	1.8	g
KCl	0.55	g
Distilled water	1.0	L
pH	7.6	

65. ISP 4 (Inorganic salts starch agar)

HiMedia No. M359

66. YPM medium

Yeast extract	5.0	g
Peptone	3.0	g
Mannitol	25.0	g
Agar	12.0	g

Distilled water 1000 ml

pH not adjusted

67. ATCC medium No. 697 (Thermus medium)

Yeast extract	4.0 g
Polypeptone peptone (BD 211910)	8 g
NaCl	2 g
Agar	30 g

Distilled water 1000 ml

Adjust the pH to 7.5

68. X medium

$MgSO_4$ $7H_2O$	0.5 g
Diammonium hydrogen phosphate	0.5 g
NaCl	3.0 g
K_2HPO_4	1.0 g
Soyabean meal	10 g
$CaCO_3$	3 g
Glycerol	15 ml

Distilled water 1000 ml

pH 7

69. Nutrient agar in artificial sea water

Beef extract	3.0	g
Peptone	5.0	g
Artificial sea water*	1.0	L
Agar	15.0	g

* Composition of artificial sea water

NaCl	23.6 g
KCl	0.64 g
$MgCl_2$ $6H_2O$	4.53 g
$MgSO_4$ $7H_2O$	5.94 g
$CaCl_2$ $2H_2O$	1.30 g

Distilled water 1000 ml

pH not adjusted

70. Malt extract agar

Malt extract	30 g
Peptone	5 g
Artificial Sea water	1000 ml
pH not adjusted	
Agar	15 g

71. Halodurans medium

K_2HPO_4	0.25 g
NaCl	10 g
$MgSO_4$	0.03
Glucose	10 g
Yeast extract	5 g
Peptone	10 g
Distilled water	1000 ml
Na_2CO_3	20 g (Separately autoclaved and added to the medium)

72. 2XYT medium

Tryptone	16 g
Yeast extract	10 g
NaCl	10 g
Distilled water	1000 ml
pH 9	

REVIVING FREEZE – DRIED CULTURES

Freeze dried cultures are supplied in vacuum-sealed glass ampoules. To revive the cultures, follow procedures described below and refer to the illustrations.

1. Prepare the specified liquid growth medium.
2. Score the middle of the ampoule with an ampoule cutter (Figure 1)
3. Disinfect the ampoule with alcohol-damped gauze (Figure 2).
4. Wrap sterile gauze around the ampoule and break it carefully (Figure 3 & 4).
5. Add about 0.2 ml of liquid medium with sterile Pasteur pipette (Figure 5). Mix well and transfer the cell suspension to growth medium (broth, agar slant or plate).
6. Incubate he cells in the medium under specified conditions. Since some cells may exhibit a prolonged lag period before growth, incubation should be continued for at least 2 weeks at appropriate temp. before discarding the cells as nonviable.
7. The ampoule should be kept in a refrigerator if it will not be opened soon after receipt.
8. The ampoule & any remaining contents should be sterilized before discarding.

INSTRUCTIONS FOR OPENING OF AMPOULES & REVIVAL OF FREEZE – DRIED CULTURES