

1567. METHYLOPROFUNDUS SEDIMENTI MEDIUM

Solution 1 (10x NMS salts):

KNO ₃	10.00	g
MgSO ₄ x 6 H ₂ O	10.00	g
CaCl ₂ x 2 H ₂ O	2.00	g
Distilled water	1000.00	ml

Dissolve the ingredients listed above (in that order) in about 700 ml of distilled water, and then make up to 1 litre.

Solution 2 Phosphate buffer (10x)

Na ₂ HPO ₄ x 12 H ₂ O	71.60	g
KH ₂ PO ₄	26.00	g
Distilled water	1000.00	ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Solution 3 Trace element solution (medium 141)

Nitilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
KAl(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Distilled water	1000.00	ml

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

Solution 4 Vitamin solution (medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H ₂ O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Continued on next page(Fe EDTA):

Solution 5 Sodium chloride (15x)

NaCl	300.0	g
Distilled water	1000.0	ml

Solution 6 (Fe EDTA):

Fe EDTA	3.80	g
Distilled water	1000.00	ml

To make liquid 1x NMS

Solution 1 (NMS)	100.0	ml
Solution 2 (Phosphate buffer)	10.0	ml
Solution 3 (Trace elements)	1.0	ml
Solution 4 (vitamine solution)	1.0	ml
Solution 5 (NaCl)	66.0	ml
Solution 6 (Fe EDTA))	0.4	ml

Bring to 1 liter with distilled water.

Prepare the growth medium as follows:

Dilute 100 ml solution 1 to 1 litre with distilled water and then add 1 ml of solution 3, 66 ml solution 5 and 0.4 ml of solution 6. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 50% methane to the gas phase and autoclave at 121°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. Filter sterilized the vitamin solution. When the growth medium is cool 1.0 ml/l vitamin solution and 10 ml/l of the phosphate buffer are added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.