

1267a: MARIPROFUNDUS (ES) MEDIUM

Solution A	901.00	ml
Solution B	1.00	ml
Solution C	100.00	ml

1. Preparation of the bottom layer: Mix 1 volume of solution A (except NaHCO₃ and agarose) with 1 volume of solution C and add 1% (w/v) agarose type 1, low EEO. After autoclaving, aseptically fill 1.2 ml of the suspension in sterile screw capped tubes (10 ml total volume). The bottom layer solidifies in approx. 30 min.

2. Preparation of the top layer: Add bicarbonate and low melt agarose to solution A and autoclave. Let the sterile solution cool to 40°C and add 10.00 ml/l of solution B. Sparge solution with sterile CO₂ gas until a pH of 6.1 - 6.4 is reached. Then, aseptically pipette aliquots of 6.0 ml over the bottom layer of each tube and let medium equilibrate for at least three hours, but not longer than 12 hours.

3. Inoculation: Inoculate the semisolid top layer with a pipette that is inserted just above the bottom layer; the pipette tip is drawn upward as the inoculum is dispensed.

Solution A

NaCl	27.50	g
MgCl ₂ x 6 H ₂ O	5.38	g
MgSO ₄ x 7 H ₂ O	6.78	g
KCl	0.72	g
CaCl ₂ x 2 H ₂ O	1.40	g
NH ₄ Cl	1.00	g
K ₂ HPO ₄	0.05	g
Wolfe's mineral elixir	1.00	ml
NaHCO ₃ (for top layer)	0.50	g
Agarose (for top layer, low melt)	1.50	g
Distilled water	900.00	ml

Solution B

Wolin's vitamin solution (10x)	1.00	ml
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Solution C

Ferrous sulfide sludge	100.00	ml
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Wolfe's mineral elixir (from medium 792)

MgSO ₄ x 7 H ₂ O	30.00	g
MnSO ₄ x H ₂ O	5.00	g

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NaCl	10.00	g
FeSO ₄ x 7 H ₂ O	1.00	g
CoCl ₂ x 6 H ₂ O	1.80	g
CaCl ₂ x 2 H ₂ O	1.00	g
ZnSO ₄ x 7 H ₂ O	1.80	g
CuSO ₄ x 5 H ₂ O	0.10	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.18	g
H ₃ BO ₃	0.10	g
Na ₂ MoO ₄ x 2 H ₂ O	0.10	g
(NH ₄) ₂ Ni(SO ₄) ₂ x 6 H ₂ O	2.80	g
Na ₂ WO ₄ x 2 H ₂ O	0.10	g
Na ₂ SeO ₄	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H₂SO₄, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Ferrous sulfide sludge (from medium 1267)

FeSO ₄ x 7 H ₂ O	15.40	g
Na ₂ S x 9 H ₂ O	12.30	g
Distilled water	100.00	ml

Heat distilled water to 50°C in a 250 ml beaker with a stir bar present. While rapidly stirring the water, add the ferrous sulfate followed immediately by the sodium sulfide. The formed black FeS sludge is decanted into a glass bottle that can be stoppered. The FeS is allowed to settle for several hours and then the overlying water is decanted and replaced. This procedure is repeated at least five times to wash the FeS. After washing, the pH of the FeS solution should be close to neutrality. The FeS suspension can be kept in closed bottles or tubes under a nitrogen atmosphere for at least three months.