

1267. MARIPROFUNDUS (ES) MEDIUM

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 (see medium 792)	1.00	ml
NaHCO ₃ (for top layer)	0.50	g
Low melt agarose (for top layer)	1.50	g
Distilled water	900.00	ml

Solution B:

Vitamin solution (see medium 141)	10.00	ml
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Sterilize by filtration and store at 4°C in the dark.

Solution C:

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.

Preparation of the bottom layer: Mix 1 volume of *solution A* with 1 volume of *solution C* and add 1% (w/v) agarose. 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.

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.

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.