

1534. MARINIFILUM MEDIUM

NaCl	13.00	g
MgCl ₂ x 6H ₂ O	2.80	g
KCl	0.36	g
NH ₄ Cl	0.25	g
KH ₂ PO ₄	0.20	g
CaCl ₂ x2H ₂ O	0.70	g
Na ₂ SO ₄	1.42	g
Glucose	2.00	g
Resazurin (0.1%)	1.00	ml
Trace mineral element solution (SL10 medium 320)(see below)	1.00	ml
Selenite-tungstate solution (see below)	1.00	ml
NaHCO ₃	2.52	g
Na ₂ S x 9 H ₂ O	0.50	g
Vitamin solution V7 (Pfennig et al.1981) (see below)	1.00	ml
Distilled water	1000.00	ml

pH 7.0- 7.2

Prepare the medium without NaHCO₃, Na₂S x 9 H₂O, vitamin solution, Selenite-tungstate solution and glucose. Boil the medium in a water bath and cool under a stream of N₂/CO₂ (80/20). Add the NaHCO₃ to the cooled medium (the pH should be 7.0-7.2) and dispense the medium into Hungate tubes or serum bottles under a stream of N₂/CO₂ (80/20). Seal the tubes under N₂/CO₂ (80/20) and autoclave. To the cooled medium at the appropriate amounts of Na₂S x 9 H₂O, vitamin solution, Selenite-tungstate solution and glucose from sterile, anaerobic stock solutions.

Selenite-tungstate solution:

Na ₂ SeO ₃ x5H ₂ O	3.0	mg
Na ₂ WO ₄ x 2H ₂ O	4.0	mg
NaOH	0.5	g
Distilled water	1000	ml

Trace element solution SL-10:

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

First dissolve FeCl_2 in the HCl, then dilute in water, add and dissolve the other salts.
Finally make up to 1000.0 ml.
Adjust pH to 6.0.

Vitamin solution V7:

Biotin	20.0	mg
p-Aminobenzoic acid	50.0	mg
Vitamin B ₁₂	50.0	mg
Thiamine-dichlorid	50.0	mg
Nicotinic acid	100.0	mg
Ca-D-(+)-Panthothenic acid	25.0	mg
Pyridoxymine-Dihydrochloride	250.0	mg
Distilled water	1000.0	ml