

120d: METHANOSARCINA MARINE MEDIUM

Final pH: 7.0

K ₂ HPO ₄	0.35	g
KH ₂ PO ₄	0.23	g
NH ₄ Cl	0.50	g
MgCl ₂ x 6 H ₂ O	10.00	g
CaCl ₂ x 2 H ₂ O	0.15	g
NaCl	23.00	g
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
<i>Trace element solution SL-10</i>	1.00	ml
Na-resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
<i>Vitamin solution</i>	10.00	ml
Methanol	10.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N₂ gas atmosphere as concentrated solutions in tightly closed tubes. Carbonate is prepared under 80% N₂ and 20% CO₂ gas mixture and autoclaved separately. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

Vitamin solution (from medium 141)

Biotin	2.0	mg
Folic acid	2.0	mg
Pyridoxine hydrochloride	10.0	mg
Thiamine-HCl	5.0	mg
Riboflavin	5.0	mg
Nicotinic acid	5.0	mg
D-Ca-pantothenate	5.0	mg
Vitamin B ₁₂	0.1	mg
p-aminobenzoic acid	5.0	mg
Lipoic acid	5.0	mg
Distilled water	1000.0	ml

Trace element solution SL-10 (from medium 320)



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 FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.