

## 120d: METHANOSARCINA MARINE MEDIUM

Final pH: 6.8 - 7.0

Final volume: 1024 ml

K <sub>2</sub> HPO <sub>4</sub>	0.35	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
NH <sub>4</sub> Cl	0.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	10.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
NaCl	23.00	g
<b>FeSO<sub>4</sub> x 7 H<sub>2</sub>O solution (0.1% w/v)</b>	2.00	ml
<b>Trace element solution SL-10</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Methanol (50% v/v)	20.00	ml
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas atmosphere as concentrated solutions in tightly closed tubes. Carbonate is prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and autoclaved separately. Vitamins are prepared under 100% N<sub>2</sub> 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.

### **FeSO<sub>4</sub> x 7 H<sub>2</sub>O solution (0.1% w/v)** (from medium 119)

FeSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	g
H <sub>2</sub> SO <sub>4</sub> (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

### **Trace element solution SL-10** (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g

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ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

### **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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml