

**120d. METHANOSARCINA MARINE MEDIUM**

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
FeSO <sub>4</sub> x 7 H <sub>2</sub> O solution (0.1% w/v in 0.1 N H <sub>2</sub> SO <sub>4</sub> )	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	2.00	g
Vitamin solution (see medium 141)	10.00	ml
Methanol	10.00	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 bicarbonate, 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. Bicarbonate 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.