

## 120. METHANOSARCINA 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
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
NaCl	2.25	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
Yeast extract (OXOID)	2.00	g
Casitone (BD BBL)	2.00	g
Na-acetate	2.50	g
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. Then add and dissolve bicarbonate, adjust pH to 6.8 and dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials 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. 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.

For [DSM 1825](#), [DSM 7081](#), and [DSM 11855](#) supplement medium with 5% (v/v) clarified rumen fluid (see medium 1310) and adjust pH to 6.5 – 6.8.

For [DSM 7058](#) and [DSM 9195](#) replace methanol with 5.00 g/l trimethylamine-HCl.

For [DSM 21571](#) supplement medium with 6.00 g/l NaCl.