

## 317: METHANOSARCINA THERMOPHILA MEDIUM

NH <sub>4</sub> Cl	1.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.20	g
<b>Minerals solution</b>	50.00	ml
<b>Modified Wolin's mineral solution</b>	10.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Methanol	5.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	950.00	ml

Dissolve ingredients except carbonate, methanol, cysteine and sulfide, then 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 the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add methanol, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Prior to use adjust pH of complete medium to 6.8 - 7.0, if necessary.

### Minerals solution (from medium 317)

KH <sub>2</sub> PO <sub>4</sub>	6.00	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	6.00	g
NaCl	12.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	2.60	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.16	g
Distilled water	1000.00	ml

### Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g

H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.