

## 318. METHANOSARCINA THERMOPHILA (BCYT) MEDIUM

KH <sub>2</sub> PO <sub>4</sub>	0.30	g
NaCl	0.60	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.08	g
Trace element solution (see next page)	10.00	ml
NH <sub>4</sub> Cl	1.00	g
Yeast extract (OXOID)	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
KHCO <sub>3</sub>	2.00	g
Vitamin solution (see medium 141)	10.00	ml
Methanol	5.00	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, 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 vitamins (sterilized by filtration), methanol, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate 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, if necessary.

*Continued next page*

*Trace element solution:*

Nitrilotriacetic acid (NTA)	12.80	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.00	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.10	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnCl <sub>2</sub>	0.10	g
CuCl <sub>2</sub>	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	0.03	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
NaCl	1.00	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.