

**375. METHANOHALOBIUM MEDIUM**

NaCl	250.00	g
NH <sub>4</sub> Cl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
KCl	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.33	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	4.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	2.50	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract (OXOID)	0.05	g
Trimethylamine-HCl	5.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, trace elements, yeast extract, trimethylamine and sulfide. Sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for at least 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trace elements, yeast extract, trimethylamine 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. Adjust pH of complete medium to 7.4 with an anoxic sterile solution of 5% w/v Na<sub>2</sub>CO<sub>3</sub>, if necessary.

*Note: A white precipitate that forms after autoclaving of the mineral base will dissolve again after storage at room temperature for 2 – 3 days.*