

## 479. METHANOHALOPHILUS MEDIUM

NaCl	87.00	g
KCl	1.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	6.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.40	g
NH <sub>4</sub> Cl	1.00	g
K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	0.40	g
Trace element solution (see medium 141)	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Trimethylamine-HCl	2.00	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.20	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, trimethylamine, coenzyme M and sulfide. 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 dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trimethylamine, coenzyme M 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 7.0 - 7.2, if necessary.

For [DSM 5219](#) supplement medium with 0.50 g/l Casamino acids (DIFCO), 10.00 ml/l of fatty acid mixture (see medium 119) and 0.25 g/l L-cysteine-HCl x H<sub>2</sub>O from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 7.4 - 7.5 with a sterile anoxic stock solution of NaOH.