

## 525a. METHANOHALOPHILUS (MG+NaCl) MEDIUM

KCl	0.34	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	2.75	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.45	g
NH <sub>4</sub> Cl	0.25	g
K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
NaCl	150.00	g
Trace element solution (see medium 141)	10.00	ml
Na-acetate	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	4.00	g
Na <sub>2</sub> CO <sub>3</sub>	0.50	g
Trimethylamine-HCl	5.00	g
Vitamin solution (see medium 141)	10.00	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	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonates, trimethylamine, vitamins, 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 same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete medium by adding sterile anoxic stock solutions of trimethylamine, vitamins (sterilized by filtration), cysteine and sulfide prepared under 100% N<sub>2</sub> gas atmosphere and bicarbonate and carbonate from sterile anoxic stock solutions prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of complete medium to 6.9 - 7.0, if necessary.