

713. METHANOHALOPHILUS EUHALOBIUS MEDIUM

NH ₄ Cl	0.5	g
NaCl	60.0	g
MgCl ₂ x 6 H ₂ O	2.4	g
KCl	1.0	g
KH ₂ PO ₄	0.4	g
Na ₂ -EDTA	0.5	g
Yeast extract	2.0	g
Trace element solution (see medium 141)	10.0	ml
Na-resazurin solution (0.1% w/v)	0.5	ml
Na ₂ CO ₃	1.0	g
Trimethylamine-HCl	10.0	g
Vitamin solution (see medium 141)	5.0	ml
CaCl ₂ x 2 H ₂ O	2.0	g
L-Cysteine-HCl x H ₂ O	0.5	g
Na ₂ S x 9 H ₂ O	0.5	g
Distilled water	1000.0	ml

Dissolve ingredients (except carbonate, trimethylamine, vitamins, calcium chloride, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 80% N₂ and 20% CO₂ gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add trimethylamine, calcium chloride, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.