713. METHANOHALOPHILUS EUHALOBIUS MEDIUM

NH₄Cl 0.5 g
NaCl 60.0 g
MgCl₂ x 6 H₂O 2.4 g
KCl 1.0 g
KH₂PO₄ 0.4 g
Na₂-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
Na₂CO₃ 1.0 g
Trimethylamine-HCl 10.0 g
Vitamin solution (see medium 141) 5.0 ml
CaCl₂ x 2 H₂O 2.0 g
L-Cysteine-HCl x H₂O 0.5 g
Na₂S x 9 H₂O 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.