713. METHANOHALOPHILUS EUHALOBIUS MEDIUM

NH_4Cl	0.5	g
NaCl	60.0	g
$MgCl_2$ x 6 H_2O	2.4	g
KCl	1.0	g
KH_2PO_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
$NaHCO_3$	4.0	g
Trimethylamine-HCl	10.0	g
Vitamin solution (see medium 141)	5.0	ml
$CaCl_2$ x 2 H_2O	2.0	g
L-Cysteine-HCl x H_2O	0.5	g
Na_2S x 9 H_2O	0.5	g
Distilled water	1000.0	ml

Dissolve ingredients (except bicarbonate, trimethylamine, vitamins, calcium chloride, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 80% N_2 and 20% CO_2 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% N_2 gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.