

329. METHANOHALOPHILUS HALOPHILUS MEDIUM

NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
KCl	0.33	g
K ₂ SO ₄	0.01	g
NaCl	70.00	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract (OXOID)	0.05	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	5.00	g
MgCl ₂ x 6 H ₂ O	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
Vitamin solution (see medium 141)	10.00	ml
Trimethylamine-HCl	5.00	g
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, magnesium chloride, calcium chloride, vitamins, trimethylamine, cysteine and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add bicarbonate and equilibrate pH to 6.9 - 7.0, then distribute under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add magnesium chloride, calcium chloride, vitamins (sterilized by filtration), trimethylamine, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use adjust pH of complete medium to 7.2, if necessary.