

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	1.00	ml
Yeast extract (OXOID)	0.05	g
Sodium resazurin (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
Trimethylamine-HCl	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
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.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml