375: METHANOHALOBIUM MEDIUM

NaCl	250.00	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$MgSO_4$ x 7 H_2O	4.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	0.05	g
Trimethylamine-HCl	5.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, trace elements, yeast extract, trimethylamine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trace elements, yeast extract, trimethylamine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.4 with an anoxic sterile solution of 5% w/v Na_2CO_3, if necessary.

2. Note: A white precipitate that forms after autoclaving of the mineral base will dissolve again after storage at room temperature for 2 - 3 days.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.