905. METHANOCALCULUS HALOTOLERANS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
KCl	0.17	g
NaCl	50.00	g
Na-acetate	0.50	g
Yeast extract (OXOID)	0.50	g
Trypticase (BD BBL)	0.50	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
L-Cysteine-HCl x H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.60	g
$MgCl_2$ x 6 H_2O	3.20	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, cysteine, calcium chloride, magnesium chloride and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Add and dissolve solid bicarbonate and cysteine, then adjust pH to 7.5 with 10 N KOH. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (to 30% of volume) and autoclave. Add calcium chloride, magnesium chloride and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The pH of the complete medium should be between 7.2 and 7.6. After inoculation pressurize vessels with sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.