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
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (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

1. 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.

2. After inoculation pressurize vessels with sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.