ATCC medium: 1343 General salts medium for estuarine methanogens

Complete Salts Solution (see below).....200.0 ml
Wolfe's Mineral Solution (see below).....4.0 ml
Wolfe's Vitamin Solution (see below).....4.0 ml
20% Yeast Extract - Trypticase Solution (see below)....4.0 ml
0.1% Resazurin.....0.4 ml
25% Sodium acetate solution.....2.0 ml
0.2% $Fe(NH_4)_2SO_4$ solution.....0.4 ml
$NaHCO_3$.....2.0 g
NaCl.....3.6 g
Cysteine-Sulfide Reducing Agent (see below).....16.0 ml
Agar (for solid medium).....8.0 g
Distilled water.....180.0 ml

Adjust medium for final pH of 7.0. Bring to boil under 80% N_2, 20% CO_2 and add reducing agent. Wait until reduced; stopper with butyl rubber and dispense anaerobically into aluminum seal-type culture tubes (Bellco #2048-18150). Autoclave at 121C for 15 minutes.

Complete Salts Solution:
KCl.....0.67 g
$MgCl_2 . 6H_2O$.....5.5 g
$MgSO_4 . 7H_2O$.....6.9 g
NH_4Cl.....0.5 g
$CaCl_2 . 2H_2O$.....0.28 g
K_2HPO_4.....0.28 g
Distilled water.....1.0 L

Wolfe's Vitamin Solution:
Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).
Biotin.....2.0 mg
Folic acid.....2.0 mg
Pyridoxine hydrochloride.....10.0 mg
Thiamine . HCl.....5.0 mg
Riboflavin.....5.0 mg
Nicotinic acid.....5.0 mg
Calcium D-(+)-pantothenate.....5.0 mg
Vitamin B12.....0.1 mg
p-Aminobenzoic acid.....5.0 mg
Thioctic acid.....5.0 mg
Distilled water.....1.0 L

Wolfe's Mineral Solution:
Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....1.5 g
$MgSO_4 . 7H_2O$ .....3.0 g
$MnSO_4 . H_2O$ .....0.5 g
NaCl.....1.0 g
$FeSO_4 . 7H_2O$ .....0.1 g
$CoCl_2 . 6H_2O$ .....0.1 g
$CaCl_2$.....0.1 g
$ZnSO_4 . 7H_2O$ .....0.1 g
$CuSO_4 . 5H_2O$ .....0.01 g
$AlK(SO_4)_2 . 12H_2O$.....0.01 g
H_3BO_3 .....0.01 g
$Na_2MoO_4 . 2H_2O$.....0.01 g
Distilled water.....1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

20% Yeast Extract - Trypticase Solution:

Yeast extract.....20.0 g
Trypticase Peptone (BD 211921)....20.0 g
Distilled water to.....100.0 ml

Cysteine-Sulfide Reducing Agent:

N NaOH (4 g/100 ml).....30.0 ml
L-Cysteine . HCl.....5.0 g
12.5% Na_2S stock solution (freshly prepared) .....40.0 ml
Distilled water.....330.0 ml

Add distilled water to 500 ml round-bottom flask. Add fresh NaOH; bring to boil under N_2. Remove gassing probe, add cysteine (swirling to dissolve), then Na_2S solution. Renew gassing for several minutes; seal with butyl rubber stoppers. Transfer to hood and dispense into 8.0 ml/18 mm Hungate tubes.