

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) ₂ SO 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% N₂, 20% CO₂ 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 ₂ O	5.5 g
MgSO ₄ . 7H ₂ O	6.9 g
NH ₄ Cl	0.5 g
CaCl ₂ . 2H ₂ O	0.28 g
K ₂ HPO 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 ₂ O	3.0 g
MnSO ₄ . H ₂ O	0.5 g
NaCl.....	1.0 g
FeSO ₄ . 7H ₂ O	0.1 g
CoCl ₂ . 6H ₂ O	0.1 g
CaCl ₂	0.1 g
ZnSO ₄ . 7H ₂ O	0.1 g
CuSO ₄ . 5H ₂ O	0.01 g
AlK(SO ₄) ₂ . 12H ₂ O.....	0.01 g
H ₃ BO ₃	0.01 g
Na ₂ MoO ₄ . 2H ₂ O.....	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 ₂ S 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 N₂. Remove gassing probe, add cysteine (swirling to dissolve), then Na₂S solution. Renew gassing for several minutes; seal with butyl rubber stoppers. Transfer to hood and dispense into 8.0 ml/18 mm Hungate tubes.