

ATCC medium: 1340 MS medium for methanogens

Mineral Solution #1 (see below).....	15.0 ml
Mineral Solution #2 (see below).....	15.0 ml
20% Yeast Extract - Trypticase Solution (see below) ..	4.0 ml
20% Sodium formate.....	6.0 ml
25% Sodium acetate.....	4.0 ml
0.2% FeSO ₄ . 7H ₂ O	0.4 ml
0.1% Resazurin.....	0.4 ml
Wolfe's Vitamin Solution (see below).....	4.0 ml
Wolfe's Mineral Solution (see below).....	4.0 ml
NaHCO ₃	2.4 g
Agar (if needed).....	8.0 g
Cysteine-Sulfide Reducing Agent (see below).....	16.0 ml
Distilled water.....	340.0 ml

Bring to boil under 80% N₂, 20% CO₂. Wait until reduced. Stopper with butyl rubber. Dispense under same gas phase into anaerobic aluminum seal type culture tubes (Bellco #2048-18150). Adjust final pH to 7.0. Autoclave at 121C for 15 min.

Mineral Solution #1:

K ₂ HPO ₄	6.0 g
Distilled water.....	1.0 L

Mineral Solution #2:

KH ₂ PO ₄	6.0 g
(NH ₄) ₂ SO ₄	6.0 g
NaCl.....	12.0 g
MgSO ₄ . 7H ₂ O	2.6 g
CaCl ₂ . 2H ₂ O	0.16 g
Distilled water.....	1.0 L

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.

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
MgSO₄ . 7H₂O3.0 g
MnSO₄ . H₂O0.5 g
NaCl.....1.0 g
FeSO₄ . 7H₂O0.1 g
CoCl₂ . 6H₂O0.1 g
CaCl₂0.1 g
ZnSO₄ . 7H₂O0.1 g
CuSO₄ . 5H₂O0.01 g
AlK(SO₄)₂ . 12H₂O.....0.01 g
H₃BO₃0.01 g
Na₂MoO₄ . 2H₂O.....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.