

ATCC medium: 1275 *Haloanaerobium* medium

NaCl.....130.0 g
MgSO₄ . H₂O5.0 g
KCl.....1.0 g
Yeast extract.....10.0 g
Trypticase.....10.0 g
Wolfe's Vitamin Solution (see below).....10.0 ml
Wolfe's Mineral Solution (see below).....10.0 ml
Distilled water.....1.0 L

Tube under 97% N₂, 3% H₂, 10 ml/tube. After autoclaving and before inoculation, add aseptically:

Thioglycollate-Ascorbate Reducing Agent (see below)..0.25 ml
Glucose (10%).....0.25 ml
2 N NaOH.....0.1 ml

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.

Thioglycollate - Ascorbate Reducing Agent:

Sodium thioglycollate.....	1.0 g
Ascorbic acid.....	1.0 g
Distilled water.....	100.0 ml

Adjust pH to 7.0 and filter-sterilize.