

ATCC medium: 2245 *Thermoanaerobacterium* medium

Trypticase.....0.25 g
Raffinose.....5.0 g
Pfennig's Mineral Solution (see below)..50.0 ml
Wolfe's Mineral Solution (see below).....1.0 ml
Resazurin (0.1% w/v).....1.0 ml
Distilled water.....950.0 ml

Combine above ingredients. Autoclave at 121C for 15 minutes. Cool under N₂ gas.

Add, in order given, the following filter-sterilized solutions:

NaHCO₃ (7%).....70.0 ml
Wolfe's Vitamin Solution (see below).....5.0 ml
L-Cysteine . HCl (1.25%).....20.0 ml
N₂S . 9H₂O (1.25%)20.0 ml

Adjust final pH to 6.8 +/- 0.2. Dispense anaerobically in 10.0-ml amounts into rubber-stoppered test tubes.

Pfennig's Mineral Solution:

KH₂PO₄10.0 g
MgCl₂ . 6H₂O6.6 g
NaCl.....8.0 g
NH₄Cl8.0 g
CaCl₂ . 2H₂O1.0 g
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.

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