

ATCC medium: 1777 *Ferrihydrobacterium* medium

Prepare ATCC Medium 1465 (see below) anaerobically as a single solution and reduce sodium sulfide to 0.5 g/L. Adjust medium for final pH 7.0 with H₂SO₄ and autoclave at 121C for 15 minutes.

ATCC Medium 1465:

Solution 1:

NH ₄ Cl	0.9 g
MgCl ₂ . 6H ₂ O	0.2 g
KH ₂ PO ₄	0.75 g
K ₂ HPO ₄	1.5 g
Trace Element Solution (see below).....	9.0 ml
10% FeSO ₄ . 7H ₂ O	0.03 ml
0.2% Resazurin.....	1.0 ml
Wolfe's Vitamin Solution (see below).....	5.0 ml
Trypticase Peptone (BD 211921).....	10.0 g
Distilled water.....	850.0 ml

Solution 2:

Yeast extract.....	3.0 g
Distilled water.....	100.0 ml

Solution 3:

Glucose.....	5.0 g
Distilled water.....	50.0 ml

Autoclave the above solutions separately at 121C for 45 minutes. Combine solutions under 100% N₂ and dispense aseptically using the same gas phase. At time of inoculation add 10% sterile Na₂S . 9H₂O to a final concentration of 0.1%.

Trace Element Solution:

Nitrilotriacetic acid (Adjust to pH 6.5 with KOH).....	12.5 g
FeCl ₃ . 4H ₂ O	0.2 g
MnCl ₂ . 4H ₂ O	0.1 g
CoCl ₂ . 6H ₂ O	0.017 g
CaCl ₂ . 2H ₂ O	0.1 g
ZnCl ₂	0.1 g
CuCl ₂	0.02 g
H ₃ BO ₃	0.01 g
Na ₂ MoO ₄ . 2H ₂ O.....	0.01 g
NaCl.....	1.0 g
Na ₂ SeO ₃	0.02 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