

ATCC medium: 1797 Coal medium

NH ₄ Cl	0.4 g
NaCl.....	0.4 g
MgCl ₂ . 6H ₂ O	0.33 g
CaCl ₂ . 2H ₂ O	0.05 g
KH ₂ PO ₄	1.0 mg
Trace Elements Solution SL-10 (see below).....	1.0 ml
Na ₂ SeO ₃ . 5H ₂ O.....	3.0 mcg
Wolfe's Vitamin Solution (see below).....	10.0 ml
Yeast extract.....	2.0 g
Resazurin.....	1.0 mg
Pittsburgh Seam Coal.....	10.0 g
NaHCO ₃	3.5 g
Na ₂ S . 9H ₂ O	0.3 g
Distilled water.....	1.0 L

Prepare medium anaerobically using Hungate techniques under 80% N₂, 20% CO₂. Autoclave at 121C for 15 minutes. Final pH 7.3 +/- 0.1.

Trace Elements Solution SL-10:

HCl (25%).....	10.0 ml
FeCl ₂ . 4H ₂ O	1.5 g
ZnCl ₂	70.0 mg
MnCl ₂ . 4H ₂ O	100.0 mg
H ₃ BO ₃	6.0 mg
CoCl ₂ . 6H ₂ O	190.0 mg
CuCl ₂ . 2H ₂ O	2.0 mg
NiCl ₂ . 6H ₂ O	24.0 mg
Na ₂ MOO ₄ . 2H ₂ O.....	36.0 mg
Distilled water.....	990.0 ml

Dissolve FeCl₂ in the HCl, dilute with water, add and dissolve the other salts, adjust pH to 6.0 with NaOH, and fill up to 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