

ATCC medium: 2172 BA medium (DSM 671)

HN ₄ Cl	1.0 g
NaCl.....	0.1 g
MgCl ₂ . 6H ₂ O	0.1 g
CaCl ₂ . 2H ₂ O	0.05 g
K ₂ HPO ₄ . 3H ₂ O.....	0.4 g
Resazurin.....	0.5 mg
Modified Wolfe's Minerals (see below)...	10.0 ml
NaHCO ₃	2.6 g
Yeast extract.....	0.75 g
Cellobiose.....	4.0 g
(<u>or</u> Cellulose-Avicel, 2.0 g)	
Wolfe's Vitamins (see below).....	10.0 ml
Na ₂ S . 9H ₂ O	0.25 mg
Distilled water.....	1.0 L

Prepare base medium under 80% N₂, 20% CO₂. Autoclave at 121C for 15 minutes. Separately sterilize stock solutions of cellobiose (autoclave or filter), vitamins (filter), and sodium sulfide (autoclave), then add them to the sterile base medium. Adjust pH to 6.9-7.0. Dispense under 80% N₂, 20% CO₂.

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

Modified Wolfe's Minerals:

Na ₂ SeO ₃	10.0 mg
NiCl ₂ . 6H ₂ O	10.0 mg
Na ₂ WO ₄ . 2H ₂ O.....	10.0 mg
Wolfe's Mineral Solution (see below).....	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.