

ATCC medium: 2193 RV5 medium

EDTA (1%).....	1.0 ml
MgSO ₄ . 7H ₂ O (20%)	0.5 ml
CaCl ₂ . 2H ₂ O	0.1 ml
NaCl.....	15.0 g
NH ₄ Cl.....	0.52 g
Sodium malate (10%).....	40.0 ml
Vitamin B ₁₂	20.0 mcg
Thiamine.....	1.0 mg
Biotin.....	30.0 mcg
Wolfe's Mineral Solution (see below).....	1.0 ml
Yeast extract.....	0.5 g
K ₂ HPO ₄	1.0 g
BICINE buffer.....	10.0 g
Distilled water.....	960.0 ml

Adjust pH to 9.3. Autoclave for 20 minutes at 121C.

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.