

ATCC medium: 2294 MPOB medium

Na ₂ HPO ₄ . 2H ₂ O.....	0.53 g
KH ₂ PO ₄	0.41 g
NH ₄ Cl	0.3 g
CaCl ₂ . 2H ₂ O	0.11 g
MgCl ₂ . 6H ₂ O	0.1 g
NaCl.....	0.3 g
Wolfe's Mineral Solution (see below).....	1.0 ml
Selenite/Tungstate Solution (see below).....	1.0 ml
Yeast extract.....	0.2 g
Resazurin.....	0.5 mg
NaHCO ₃	4.0 g
Wolfe's Vitamin Solution (see below).....	10.0 ml
Sodium fumarate.....	3.2 g
Na ₂ S . 9H ₂ O	0.5 g
Distilled water.....	1.0 L

Prepare the basal medium anaerobically under 80% N₂, 20% CO₂. Autoclave at 121C for 15 minutes. Prepare and outgas separate anaerobic sterile stock solutions of sodium bicarbonate (use N₂ and CO₂), vitamin solution (use N₂), sodium fumarate (use N₂), and sodium sulfide (use N₂). Combine the basal medium and stock solutions. The pH of the completed medium should be 6.9-7.1.

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.

Selenite/Tungstate Solution:

NaOH.....0.5 g
Na₂SeO₃ . 5H₂O.....3.0 mg
Na₂WO₄ . 2H₂O.....4.0 mg
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