

ATCC medium: 1754 PETC medium

NH ₄ Cl	1.0 g
KCl.....	0.1 g
MgSO ₄ . 7H ₂ O	0.2 g
NaCl.....	0.8 g
KH ₂ PO ₄	0.1 g
CaCl ₂ . 2H ₂ O	20.0 mg
Yeast extract.....	1.0 g
Trace Elements (see below).....	10.0 ml
Wolfe's Vitamin Solution (see below)....	10.0 ml
NaHCO ₃	2.0 g
Fructose.....	5.0 g
Reducing Agent (see below).....	10.0 ml
Distilled water.....	980.0 ml

Add ingredients through vitamins and adjust to pH 5.5. Add NaHCO₃, boil, degas and dispense anaerobically under 100% CO₂. Autoclave at 121C for 15 minutes. Add fructose and reducing agents from sterile anaerobic stock solutions prepared under 100% N₂. Final pH is 5.9.

Trace Elements:

Nitrilotriacetic acid.....	2.0 g
MnSO ₄ . H ₂ O	1.0 g
Fe(SO ₄) ₂ (NH ₄) ₂ . 6H ₂ O	0.8 g
CoCl ₂ . 6H ₂ O	0.2 g
ZnSO ₄ . 7H ₂ O	0.2 mg
CuCl ₂ . 2H ₂ O	20.0 mg
NiCl ₂ . 6H ₂ O	20.0 mg
Na ₂ MoO ₄ . 2H ₂ O.....	20.0 mg
Na ₂ SeO ₄	20.0 mg
Na ₂ WO ₄	20.0 mg
Distilled water.....	1.0 L

Add nitrilotriacetic acid to water and adjust to pH 6.0 with KOH. Add remainder of ingredients.

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

Reducing Agent:

NaOH.....	0.9 g
L-Cysteine . HCl.....	4.0 g
Na ₂ S . 9H ₂ O	4.0 g
Distilled water.....	100.0 ml

Boil NaOH and water under 100% N₂. Add cysteine; then Na₂S. Tube anaerobically with same gas phase and autoclave.