

**ATCC medium: 2254 MMB medium**

Peptone.....0.1 g  
Yeast extract.....0.1 g  
Glucose.....1.0 g  
Ammonium sulfate.....0.2 g  
Hutner's Basal Salts (see below).....20.0 ml  
Wolfe's Vitamin Solution (see below)....10.0 ml  
Distilled water.....1.0 L

pH 7.0-7.5

For solid medium, add 15 g of agar per liter.

*Hutner's Basal Salts:*

Nitrilotriacetic acid.....10.0 g  
MgSO<sub>4</sub>.....14.45 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....4.42 g  
Ammonium molybdate.....9.25 mg  
FeSO<sub>4</sub> . 7H<sub>2</sub>O .....99.0 mg  
Nicotinic acid.....50.0 mg  
Thiamine . HCl.....25.0 mg  
Biotin.....0.5 mg  
Metals "44" (see below).....50.0 ml  
Distilled water.....950.0 ml

Dissolve and neutralize the nitrilotriacetic acid in 500.0 ml of distilled water with the addition of KOH pellets. Add remaining chemicals in order listed. Adjust to pH 6.8. Bring volume to 1.0 L with distilled water.

*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