

**ATCC medium: 1476 TYEG medium**

NaCl.....100.0 g  
NH<sub>4</sub>Cl .....1.0 g  
KH<sub>2</sub>PO<sub>4</sub> .....0.3 g  
Na<sub>2</sub>HPO<sub>4</sub> . 7H<sub>2</sub>O.....2.1 g  
MgCl<sub>2</sub> . 6H<sub>2</sub>O .....0.2 g  
Trypticase Peptone (BD 211921).....10.0 g  
Trace Mineral Solution II (see below)...10.0 ml  
2.5% FeSO<sub>4</sub>.....25.0 mcl  
Resazurin.....2.0 mg  
Distilled water to.....960.0 ml

Adjust medium to pH 7.3, +/- 0.1. Bring to boiling under 90% N<sub>2</sub>, 10% CO<sub>2</sub> and autoclave under the same atmosphere at 121C for 15 minutes. Cool and aseptically add the following reduced filter-sterilized solutions:

10% Glucose.....50.0 ml  
10% Yeast extract.....5.0 ml  
Wolfe's Vitamin Solution (see below)....10.0 ml

At time of inoculation add 0.125 ml of 2.5% Na<sub>2</sub>S . 9H<sub>2</sub>O per 5 ml medium for a final concentration of approximately 0.06% (g/100 ml of medium).

*Trace Mineral Solution II:*

Nitrilotriacetic acid.....12.8 g  
FeSO<sub>4</sub> . 7H<sub>2</sub>O .....0.1 g  
MnCl<sub>2</sub> . 4H<sub>2</sub>O .....0.1 g  
CoCl<sub>2</sub> . 6H<sub>2</sub>O .....0.17 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....0.1 g  
ZnCl<sub>2</sub>.....0.1 g  
CuCl<sub>2</sub> . 2H<sub>2</sub>O .....0.02 g  
H<sub>3</sub>BO<sub>3</sub> .....0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> . 2H<sub>2</sub>O.....0.01 g  
NaCl.....0.1 g  
Na<sub>2</sub>SeO<sub>3</sub> .....0.017 g  
NiSO<sub>4</sub> . 6H<sub>2</sub>O .....0.026 g  
Distilled water to.....1.0 L

Add nitrilotriacetic acid to 200 ml distilled water and adjust pH to 6.5 with KOH. Add about 600 ml distilled water and dissolve the remaining components, in order, one at a time. Bring volume to 1.0 L with distilled water. Remove any brown precipitate by filtration. Store under N<sub>2</sub>.

*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