

**ATCC medium: 1515 Py-inositol (ATCC medium 1524) with 10 g *i*-inositol per liter of medium**

*ATCC Medium 1524:*

Peptone, Bacto (BD 211677).....5.0 g  
Trypticase Peptone (BD 211921).....5.0 g  
Yeast extract.....10.0 g  
*i*-Inositol.....10.0 g  
0.025% Resazurin, aqueous.....4.0 ml  
Salt Solution (see below).....40.0 ml  
Hemin Solution (see below).....10.0 ml  
Vitamin K1 Solution (see below).....0.2 ml  
L-Cysteine . HCl.....0.5 g  
Distilled water.....950.0 ml

Mix all of the ingredients except Hemin Solution, Vitamin K1, and cysteine and bring to boil under 80% N<sub>2</sub>, 10% H<sub>2</sub>, 10% CO<sub>2</sub>. After cooling, add the Hemin Solution, the Vitamin K1 Solution and the cysteine; adjust for pH of 6.9. Tube medium anaerobically using Hungate methods and the same gas phase. Autoclave at 121C for 15 minutes.

*Salt Solution:*

CaCl<sub>2</sub>.....0.2 g  
MgSO<sub>4</sub>.....0.2 g  
K<sub>2</sub>HPO<sub>4</sub>.....1.0 g  
KH<sub>2</sub>PO<sub>4</sub>.....1.0 g  
NaHCO<sub>3</sub>.....10.0 g  
NaCl.....2.0 g  
Distilled water.....1.0 L

Mix CaCl<sub>2</sub> and MgSO<sub>4</sub> in 300.0 ml distilled water until dissolved. Add 500.0 ml water and the remaining salts while swirling slowly. Add 200.0 ml distilled water and mix. Store at 4C.

*Hemin Solution:*

Hemin.....50.0 mg  
N NaOH.....1.0 ml  
Distilled water to.....100.0 ml

Suspend hemin in NaOH; then add water. Autoclave at 121C for 15 minutes.

*Vitamin K1 Solution:*

Vitamin K1.....0.15 ml  
95% Ethanol.....30.0 ml

Dissolve vitamin K1 in ethanol. Store in brown bottle at 4C. Discard after 1 month.