

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% N₂, 10% H₂, 10% CO₂. 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:

CaCl₂.....0.2 g
MgSO₄.....0.2 g
K₂HPO₄.....1.0 g
KH₂PO₄.....1.0 g
NaHCO₃.....10.0 g
NaCl.....2.0 g
Distilled water.....1.0 L

Mix CaCl₂ and MgSO₄ 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.