

ATCC medium: 1828 PY Medium (ATCC medium 1524) with cocarboxylase and serum

ATCC Medium 1524 (see below) with cocarboxylase and serum
Add cocarboxylase-serum solution to a final concentration of 10% aseptically after sterilization of PY medium.

Cocarboxylase-Serum Solution:

0.005% cocarboxylase, filter-sterilized.....1.0 ml
Heat-inactivated, sterile rabbit serum.....100.0 ml

ATCC Medium 1524:

Peptone, Bacto (BD 211677).....5.0 g
Trypticase Peptone (BD 211921).....5.0 g
Yeast extract.....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

Adjust medium for a final pH of 7.0. Mix all ingredients except cysteine and bring to boil under 80% N₂, 10% H₂, 10% CO₂. Add cysteine and re-adjust pH, if necessary. 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

Store in brown bottle at 4C. Discard after 1 month.