ATCC medium: 1237 Modified peptone-yeast extract glucose (MPYG)

Peptone........................................10.0 g
Yeast extract..................................10.0 g
Resazurin solution (0.025%)...................4.0 ml
Salt Solution (see below)......................40.0 ml
Vitamin K3-Hemin Solution (see below)........10.0 ml
L-Cysteine . HCl................................0.5 g
(NH4)2SO4......................................0.5 g
Volatile Fatty Acid Solution (see below)......3.1 ml
Glucose........................................5.0 g
Distilled water.............................887.0 ml

Bring all of the constituents except the vitamin K3-hemin solution, L-cysteine . HCl and volatile acid solution to a boil under 97% N₂, 3% H₂. After the solution has boiled and reduced, cool, add cysteine, vitamin K3-hemin solution and volatile fatty acid solution and adjust pH to 7.0 if necessary. Dispense under an atmosphere of 97% N₂, 3% H₂. Stopper with butyl rubber and autoclave in press.

Salts Solution:
CaCl₂ (anhydrous).......................0.2 g
MgSO₄ ........................................0.2 g
K₂HPO₄ ....................................1.0 g
KH₂PO₄ ...................................1.0 g
NaHCO₃ ....................................10.0 g
NaCl...........................................2.0 g

Dissolve CaCl₂ and MgSO₄ in 300 ml of distilled water. Add 500 ml water and add the remaining salts while swirling slowly. Add 200 ml of distilled water, mix, and store at 4C.

Vitamin K3-Hemin Solution:
Part A:
Menadione (Vitamin K3)...........100.0 mg
Ethanol (95%)......................30.0 ml

Add menadione to ethanol. Filter-sterilize.
Part B:
Hemin.............................50.0 mg
N NaOH.............................1.0 ml
Distilled water to.............100.0 ml

Dissolve hemin in NaOH and bring volume to 100 ml with distilled water. Autoclave solution at 121°C for 15 minutes and cool. Aseptically add 1 ml sterile menadione solution to 100 ml hemin solution. Use this vitamin K3-hemin solution at a concentration of 10 ml/L medium.

Volatile Fatty Acid Solution:
Propionic acid.....................6.0 ml
n-Butyric acid.....................4.0 ml
n-Valeric acid....................1.0 ml
Isovaleric acid...................1.0 ml
Isobutyric acid...................1.0 ml
DL-alpha-Methyl butyric acid....1.0 ml
Acetic acid.......................17.0 ml