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
$(NH_4)_2SO_4$.....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% N_2, 3% H_2. 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% N_2, 3% H_2. Stopper with butyl rubber and autoclave in press.

Salts Solution:
$CaCl_2$ (anhydrous) .....0.2 g
$MgSO_4$ .....0.2 g
K_2HPO_4 .....1.0 g
KH_2PO_4 .....1.0 g
$NaHCO_3$ .....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 121C 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