

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) ₂ SO 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% 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:

CaCl ₂ (anhydrous)	0.2 g
MgSO ₄	0.2 g
K ₂ HPO ₄	1.0 g
KH ₂ PO 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