

**ATCC medium: 1928 PY Medium (ATCC medium 1524) with horse serum and NAM**

To the sterile completed PY medium [ATCC Medium 1524 (see below)], aseptically add the following to the final concentrations indicated: 5% sterile heat-inactivated horse serum and 15 mcg/ml filter-sterilized N-acetyl muramic acid.

*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% N<sub>2</sub>, 10% H<sub>2</sub>, 10% CO<sub>2</sub>. 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:*

CaCl <sub>2</sub> .....	0.2 g
MgSO <sub>4</sub> .....	0.2 g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0 g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0 g
NaHCO <sub>3</sub> .....	10.0 g
NaCl.....	2.0 g
Distilled water.....	1.0 L

Mix CaCl<sub>2</sub> and MgSO<sub>4</sub> 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.