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% 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 121°C for 15 minutes.

Salt Solution:
CaCl₂.............................0.2 g
MgSO₄.............................0.2 g
KH₂PO₄............................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 4°C.

Hemin Solution:
Hemin.............................50.0 mg
N NaOH...........................1.0 ml
Distilled water..................100.0 ml

Suspend hemin in NaOH; then add water. Autoclave at 121°C for 15 minutes.

Vitamin K1 Solution:
Vitamin K1........................0.15 ml
95% Ethanol.......................30.0 ml

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