ATCC medium: 1257 ETSA medium

Trypticase Soy Agar (BD 211043)...40.0 g
Yeast extract..........................1.0 g
Agar...................................4.0 g
KNO₃..................................0.5 g
Sodium lactate, 60% syrup........1.3 ml
Sodium succinate...................0.5 g
Sodium formate......................0.5 g
Hemin Solution (see below).........1.0 ml
Distilled water......................924.0 ml

Autoclave the above solution at 121°C for 15 minutes. Cool to 55°C. Aseptically add the following, freshly prepared, filter-sterilized solutions in the order listed:

Menadione Solution (see below).....2.0 ml
4% L-Cysteine . HCl..................10.0 ml
0.5% Dithiothreitol (DTT).........10.0 ml
10% Glucose..........................10.0 ml
1% Sodium fumarate...............2.0 ml
4% Na₂CO₃ .........................10.0 ml
Defibrinated sheep blood.........30.0 ml

This medium solidifies very quickly and should be maintained at 50-55°C while dispensing. Aseptically tube the sterile completed medium under an anaerobic atmosphere of 80% N₂, 10% CO₂, 10% H₂. Plug the tubes with butyl rubber stoppers.

A note of caution: Hydrogen gas can be explosive in the concentration used in preparing this medium. Gas tanks should be equipped with spark arrestors.

Hemin Solution:
KOH.....................................1.12 g
95% Ethanol.........................100.0 ml
Hemin.................................200.0 mg
Distilled water......................100.0 ml

Dissolve KOH in water. Add ethanol and hemin.

Menadione Solution:
Menadione (Vitamin K₃).............50.0 mg
95% Ethanol.........................50.0 ml
Distilled water......................50.0 ml

Dissolve menadione in ethanol; then add water. Filter-sterilize solution.