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.