ATCC medium: 1257 ETSA medium

Trypticase Soy Agar (BD 211043)...40.0 g
Yeast extract.....1.0 g
Agar.....4.0 g
KNO_3.....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 121C for 15 minutes. Cool to 55C. 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% Na_2CO_3 .....10.0 ml
Defibrinated sheep blood.....30.0 ml

This medium solidifies very quickly and should be maintained at 50-55C while dispensing. Aseptically tube the sterile completed medium under an anaerobic atmosphere of 80% N_2, 10% CO_2, 10% H_2. 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.