

**ATCC medium: 1357 NOS Spirochete medium**

Brain Heart Infusion Broth (BD 237500).....12.5 g  
Trypticase (BD 211921).....10.0 g  
Yeast extract.....2.5 g  
Sodium thioglycollate.....0.5 g  
L-Cysteine . HCl.....1.0 g  
L-Asparagine.....0.25 g  
Glucose.....2.0 g  
Agar, Noble (BD 214230), for semi-solid medium.....3.0 g  
Distilled water to.....1.0 L

*Supplements:*

0.2% (w/v) Thiamine pyrophosphate.....3.0 ml  
Volatile Fatty Acids (see below).....2.0 ml  
10% Sodium bicarbonate.....20.0 ml  
Normal rabbit serum.....20.0 ml

Autoclave at 121C for 15 min.

Filter-sterilize all supplements. Prepare and dispense medium anaerobically under an atmosphere of 80% N<sub>2</sub>, 10% H<sub>2</sub>, 10% CO<sub>2</sub>.

*Volatile Fatty Acids:*

Isobutyric acid.....0.5 ml  
D,L-2-Methylbutyric acid.....0.5 ml  
Isovaleric acid.....0.5 ml  
Valeric acid.....0.5 ml  
0.1 N KOH.....100.0 ml