

ATCC medium: 1296 *Spirochaeta zuelzeri* medium

Solution 1:

KH₂PO₄0.75 g
NaH₂PO₄ . H₂O.....0.25 g
L-Cysteine . HCl.....0.5 g
Distilled water.....480.0 ml

Adjust pH to 7.2 with 5.0% (w/v) KOH.

Solution 2:

Resazurin.....1.0 mg
Yeast extract.....0.2 g
NH₄Cl.....1.0 g
MgSO₄ . 7H₂O0.5 g
CaCl₂0.02 g
FeCl₃ . 6H₂O (250 mg/L)10.0 ml
Trace Element Solution (see below).....2.0 ml
Distilled water.....480.0 ml

Solution 3:

NaHCO₃5%

Solution 4:

Glucose.....10%

Trace Element Solution:

H₃BO₃56.0 mg
ZnSO₄ . 7H₂O44.0 mg
CoCl₂ . 6H₂O20.0 mg
CuSO₄ . 5H₂O2.0 mg
MnCl₂.....2.0 mg
Na₂MoO₄ . 2H₂O.....75.0 mg
Distilled water.....100.0 ml

Autoclave Solutions 1 and 2 separately. Filter-sterilize Solutions 3 and 4 with 3 under pressure. Add 480.0 ml of Solution 1 to 480.0 ml of Solution 2 under 80% N₂, 20% CO₂. While gassing, add 20.0 ml of NaHCO₃ solution and 20.0 ml of glucose solution. Dispense anaerobically and aseptically; cap with butyl rubber stoppers. Adjust pH to 7.2.