

**ATCC medium: 1345 Anaerobic cellulolytic medium**

K<sub>2</sub>HPO<sub>4</sub> .....0.4 g  
NH<sub>4</sub>Cl .....1.0 g  
MgSO<sub>4</sub> .....0.5 g  
KCl.....0.5 g  
L-Cysteine . HCl.....0.5 g  
Cellobiose.....1.0 g  
Wolfe's Mineral Solution (see below)....20.0 ml  
Yeast extract.....1.0 g  
Resazurin.....1.0 mg  
Distilled water to.....1.0 L

Dissolve ingredients in distilled water. Boil medium under a stream of 80% N<sub>2</sub>, 10% CO<sub>2</sub>, 10% H<sub>2</sub> until the resazurin indicator is colorless. Cool medium and dispense anaerobically into 16 x 125 mm tubes using the same gas mixture.

Stopper the tubes anaerobically with butyl rubber and autoclave them for 15 minutes at 121C.

To cooled, sterile medium aseptically add the following to give the indicated final concentrations:

Na<sub>2</sub>CO<sub>3</sub> (sterilized anaerobically) .....0.1%  
Na<sub>2</sub>S . 9H<sub>2</sub>O (sterilized anaerobically) ...0.15%

Adjust final pH, if necessary to 6.9 +/- 0.1.

*Wolfe's Mineral Solution:*

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....1.5 g  
MgSO<sub>4</sub> . 7H<sub>2</sub>O .....3.0 g  
MnSO<sub>4</sub> . H<sub>2</sub>O .....0.5 g  
NaCl.....1.0 g  
FeSO<sub>4</sub> . 7H<sub>2</sub>O .....0.1 g  
CoCl<sub>2</sub> . 6H<sub>2</sub>O .....0.1 g  
CaCl<sub>2</sub>.....0.1 g  
ZnSO<sub>4</sub> . 7H<sub>2</sub>O .....0.1 g  
CuSO<sub>4</sub> . 5H<sub>2</sub>O .....0.01 g  
AlK(SO<sub>4</sub>)<sub>2</sub> . 12H<sub>2</sub>O.....0.01 g  
H<sub>3</sub>BO<sub>3</sub> .....0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> . 2H<sub>2</sub>O.....0.01 g  
Distilled water.....1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.