

**ATCC medium: 1207 BC Medium for *Acetivibrio cellulolyticus***

Mineral Solution 1 (see below).....	75.0 ml
Mineral Solution 2 (see below).....	75.0 ml
FeSO <sub>4</sub> . 7H <sub>2</sub> O Solution (see below).....	10.0 ml
Vitamin mixture (see below).....	10.0 ml
Wolfe's Mineral Solution (see below).....	10.0 ml
NaHCO <sub>3</sub> .....	2.0 g
Resazurin solution (0.1% aqueous).....	1.0 ml
Distilled water.....	810.0 ml
Alpha-Cellulose (Sigma C-6429).....	3.0 g
Cysteine-Sulfide Reducing Solution (see below).....	12.8 ml

The pH should be 7.6 at room temperature; do not adjust.  
Bring medium to boil under 10% CO<sub>2</sub> 90% N<sub>2</sub>. Cool and continue flushing.  
Add 8 ml reducing solution. Heat slightly to mix if necessary and add an additional 4.8 ml of reducing solution. Dispense 7.0 ml/tube (anaerobically) and cap.

A note of caution:

This medium contains sodium sulfide and hydrogen sulfide production will occur. Hydrogen sulfide is hazardous and this medium should be prepared in a chemical fume hood.

*Mineral Solution 1:*

K <sub>2</sub> HPO <sub>4</sub> .....	3.9 g
Distilled water.....	1.0 L

*Mineral Solution 2:*

KH <sub>2</sub> PO <sub>4</sub> .....	2.4 g
Na <sub>2</sub> SO <sub>4</sub> .....	2.5 g
NH <sub>4</sub> Cl.....	12.0 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.8 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	1.2 g
Distilled water.....	1.0 L

*FeSO<sub>4</sub> . 7H<sub>2</sub>O Solution:*

Dissolve 0.2 g FeSO<sub>4</sub> . 7H<sub>2</sub>O in 100 ml distilled water. Add 3 drops concentrated HCl.

*Vitamin Mixture:*

Biotin.....2.0 mg  
B6 (pyridoxine hydrochloride).....10.0 mg  
B1 (thiamine . HCl).....5.0 mg  
B12 (crystalline cyanocobalamin).....5.0 mg  
p-Aminobenzoic acid.....0.5 mg  
Lipoic acid (D,L-6,8-Thioctic acid).....5.0 mg  
Distilled water.....1.0 L

Store below -20 C.

*Wolfe's Mineral Solution:*

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

Nitriloacetic 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.

*Cysteine-Sulfide Reducing Solution (1.25%):*

L-Cysteine . HCl.....2.5 g  
Na<sub>2</sub>S . 9H<sub>2</sub>O .....2.5 g  
Distilled water to.....200.0 ml

Dissolve cysteine in 50 ml distilled water. Adjust to pH 10 with fresh 3 N NaOH (rapidly) and start flushing with nitrogen. Add Na<sub>2</sub>S . 9H<sub>2</sub>O. Bring total volume to 200 ml (add 150 ml but subtract the volume of 3 N NaOH and boil under nitrogen). Transfer anaerobically to tubes; stopper with butyl rubber and autoclave.