

**ATCC medium: 1612 *Acetobacterium* medium**

NH <sub>4</sub> Cl .....	1.0 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33 g
K <sub>2</sub> HPO <sub>4</sub> .....	0.45 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.1 g
Wolfe's Mineral Solution (see below)....	20.0 ml
Wolfe's Vitamin Solution (see below)....	20.0 ml
Yeast extract.....	2.0 g
Fructose (see below).....	10.0 g
Resazurin.....	1.0 mg
NaHCO <sub>3</sub> .....	10.0 g
L-Cysteine . HCl.....	0.5 g
Na <sub>2</sub> S . 9H <sub>2</sub> O .....	0.5 g
Distilled water.....	1.0 L

Mix all of the ingredients except of fructose. Equilibrate medium with 80% N<sub>2</sub>, 20% CO<sub>2</sub> gas mixture until a pH of 7.4 is reached. Tube and autoclave under the same environment. Before inoculation adjust the pH to 8.2 by adding sterile anaerobic Na<sub>2</sub>CO<sub>3</sub> (0.25 ml of 5% Na<sub>2</sub>CO<sub>3</sub> per 10 ml medium) and add sterile solution of fructose to a final concentration of 1%. For autotrophic growth omit fructose and use a gas atmosphere of 80% H<sub>2</sub>, 20% CO<sub>2</sub>.

*Wolfe's Vitamin Solution:*

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Biotin.....	2.0 mg
Folic acid.....	2.0 mg
Pyridoxine hydrochloride....	10.0 mg
Thiamine . HCl.....	5.0 mg
Riboflavin.....	5.0 mg
Nicotinic acid.....	5.0 mg
Calcium D-(+)-pantothenate...	5.0 mg
Vitamin B12.....	0.1 mg
p-Aminobenzoic acid.....	5.0 mg
Thioctic acid.....	5.0 mg
Distilled water.....	1.0 L

*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.