ATCC Medium: 1019 Acetobacterium Medium

Yeast Extract.....1.0 g
NH_4Cl.....1.0 g
$MgSO_4 \cdot 7H_2O$.....0.1 g
$KH2PO_4$.....0.4 g
K_2HPO_4.....0.4 g
Wolfe's Vitamin Solution (see below)……..10.0 ml
Wolfe's Mineral Solution (see below).....10.0 ml
Resazurin solution (0.01%).....1.0 ml
$NaHCO_3$.....3.0 g
DI Water…….....955.0 ml

Mix all of the ingredients. Bring to boil to drive oxygen out. Add 40 ml of the following Reducing Agent:

Reducing Agent
L-Cysteine x HCl.....1.25 g
$Na_2S \times 9H_2O$.....1.25 g
Distilled water.....100.0 ml

Boil water and cool under 100% N_2. Add cysteine, then sodium sulfide. Tube under same gas phase and autoclave at 121ºC.

Autoclave medium w/reducing agent at 121ºC.

After autoclaving and while the medium is still hot, bubble it with oxygen-free, 90% N_2, 10% CO_2 to maintain anaerobiosis. When the medium has cooled to about 50ºC, add 25 ml of the following fructose solution:

20% Fructose Solution
Fructose……20 g
DI Water……100 ml

Filter sterilize.

Adjust the pH of the complete medium, if necessary to 6.7, tube aseptically under anaerobic conditions and cap with butyl rubber stoppers.

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
DI Water…….....1000 ml

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
$MgSO_4$ x $7H_2O$.....3.0 g
$MnSO_4$ x H_2O.....0.5 g
NaCl.....1.0 g
$FeSO_4$ x $7H_2O$.....0.1 g
$CoCl_2$ x $6H_2O$.....0.1 g
$CaCl_2$.....0.1 g
$ZnSO_4$ x $7H_2O$…….....0.1 g
$CuSO_4$ x $5H_2O$.....0.01 g
AlK(SO) x 12HO.....0.01 g
H_3BO_3.....0.01 g
Na_2MoO_4 x $2H_2O$.....0.01 g
DI Water…….....1000 ml

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.