ATCC medium: 1669 Acetogen medium

150 mM Potassium Phosphate Buffer (see below).....20.0 ml
Salts Solution 1 (see below).....40.0 ml
Salts Solution 2 (see below).....4.0 ml
NH_4Cl.....0.2 g
Yeast Extract.....0.2 g
Trace Minerals (see below).....4.0 ml
Vitamin Solution (see below).....4.0 ml
Tungstate Solution (see below).....0.4 ml
0.1% Resazurin.....0.4 ml
Sodium bicarbonate.....2.4 g
Reducing Agent (see below).....4.0 ml
Clarified rumen fluid.....20.0 ml
Distilled water.....325.0 ml

Boil basal medium (without bicarbonate and reducing agent) under 80% N_2, 20% CO_2. Cool; add bicarbonate and reducing agent and dispense in volumes needed under N_2 and CO_2 atmosphere. Autoclave at 121C for 15 minutes. After inoculation, exchange headspace for 80% H_2, 20% CO_2.

150 mM Potassium Phosphate Buffer:
Dissolve 15.68 g K_2HPO_4 in 600 ml distilled water. Dissolve 4.72 g KH_2PO_4 in 230 ml distilled water. Mix the two solutions together and use.

Salt Solution 1:
NaCl.....1.4 g
KCl.....1.6 g
$MgSO_4 \cdot 7H_2O$.....0.2 g
Distilled water.....1.0 L

Salt Solution 2:
$CaCl_2 \cdot 2H_2O$.....0.1 g
Distilled water.....1.0 L

Trace Minerals:
Wolfe's Mineral Solution (see below) with 0.1 g $NiCl_2 \cdot 6H_2O$ and 0.01 g sodium selenite per liter.

Vitamin Solution:
Ascorbic acid.....5.0 mg
Biotin.....2.0 mg
Calcium D-(+)-pantothenate.....5.0 mg
Choline chloride.....5.0 mg
Folic acid.....2.0 mg
Lipoic acid.....5.0 mg
i-Inositol.....5.0 mg
Niacinamide.....5.0 mg
Nicotinic acid.....5.0 mg
p-Aminobenzoic acid.....5.0 mg
Pyridoxal hydrochloride.....5.0 mg
Pyridoxine hydrochloride.....10.0 mg
Riboflavin.....5.0 mg
Thiamine . HCl.....5.0 mg
Vitamin B12.....0.1 mg
Distilled water.....1.0 L

Store frozen.

Tungstate Solution:
Na_2WO_4 . $2H_2O$.....99.0 mg
Distilled water.....1.0 L

Reducing Agent:
Dispense 110 ml distilled water into a 250 ml round-bottom flask. Boil under N_2 gas for 1 minute and cool to room temperature. Add and dissolve 2.5 g L-Cysteine . HCl. Adjust to pH 9 with 5N NaOH. Add and dissolve 2.5 g (washed) Na_2S . $9H_2O$ crystals. Dispense in amounts needed. Autoclave for 10 minutes.

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
$MgSO_4$. $7H_2O$ .....3.0 g
$MnSO_4$. H_2O .....0.5 g
NaCl.....1.0 g
$FeSO_4$. $7H_2O$ .....0.1 g
$CoCl_2$. $6H_2O$ .....0.1 g
$CaCl_2$ .....0.1 g
$ZnSO_4$. $7H_2O$ .....0.1 g
$CuSO_4$. $5H_2O$ .....0.01 g
$AlK(SO_4)_2$. $12H_2O$.....0.01 g
H_3BO_3 .....0.01 g
Na_2MoO_4 . $2H_2O$.....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.