ATCC medium: 1864 *Eubacterium callanderi* medium

Clarified rumen fluid.....50.0 ml
Pfennig's Mineral Solution (see below).....50.0 ml
Trace Element Solution SL-7 (see below).....1.0 ml
Wolfe's Vitamin Solution (see below).....10.0 ml
Resazurin.....1.0 mg
Glucose.....1.8 g
$NaHCO_3$.....3.5 g
Cysteine-sulfide Reducing Agent (see below)...20.0 ml
Distilled water.....869.0 ml

Combine all ingredients except sodium bicarbonate and reducing agent. Heat to boiling and cool under an atmosphere of 80% N_2, 20% CO_2. Add sodium bicarbonate and continue gassing for 10 minutes more. Dispense anaerobically into containers using same gas phase and seal with butyl rubber stoppers. Autoclave at 121C for 15 minutes. Prior to inoculation, add reducing agent as required to yield the final concentration indicated above.

Pfennig's Mineral Solution:
KH_2PO_4.....1.0 g
$MgCl_2 \cdot 6H_2O$.....0.66 g
NH_4Cl.....0.8 g
$CaCl_2 \cdot 2H_2O$.....0.10 g
NaCl.....0.8 g
Distilled water.....100.0 ml

Trace Element Solution SL-7:
Hydrochloric acid, 25%.....10.0 ml
$FeCl_2 \cdot 4H_2O$.....1.5 g
$CoCl_2 \cdot 6H_2O$.....190.0 mg
$MnCl_2 \cdot 4H_2O$.....100.0 mg
$ZnCl_2$.....70.0 mg
H_3BO_3.....62.0 mg
$Na_2MoO_4 \cdot 2H_2O$.....36.0 mg
$NiCl_2 \cdot 6H_2O$.....24.0 mg
$CuCl_2 \cdot 2H_2O$.....17.0 mg
Distilled water.....1.0 L

Dissolve the $FeCl_2 \cdot 4H_2O$ in the concentrated HCl, then dilute.
Use 1.0 ml/L of medium.

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

Cysteine-sulfide Reducing Agent:

L-Cysteine . HCl	2.5 g
3 N NaOH	13.4 ml
Na_2S . $9H_2O$	2.5 g
Distilled water to	200.0 ml

Dissolve cysteine in 50 ml distilled water and quickly adjust to pH 10 with 3 N NaOH. Add sodium sulfide and bring volume to 200 ml with distilled water. Boil, cool, and dispense anaerobically under 100% N_2. Autoclave at 121C for 15 minutes.