

**ATCC medium: 1766 Xylan medium**

Rumen fluid.....	400.0 ml
Mineral Solution I (see below).....	25.0 ml
Mineral Solution II (see below).....	25.0 ml
Yeast extract.....	0.5 g
Peptone.....	2.0 g
Wolfe's Vitamin Solution (see below).....	10.0 ml
VFA Solution (see below).....	10.0 ml
0.01% Hemin (in 0.01 N NaOH).....	10.0 ml
Trace Elements Solution SL-6 (see below).....	1.0 ml
Xylan.....	30.0 g
Agar.....	12.0 g
L-Cysteine . HCl.....	0.25 g
Na <sub>2</sub> S . 9H <sub>2</sub> O .....	0.25 g
3.96% NaHCO <sub>3</sub> .....	40.0 ml
Distilled water.....	540.0 ml

Add ingredients except cysteine, sodium sulfide and sodium bicarbonate and boil for a few minutes to degas. Cool under 80% N<sub>2</sub>, 20% CO<sub>2</sub> and add cysteine and sodium sulfide. Add enough NaHCO<sub>3</sub> solution (freshly prepared and saturated with 100% CO<sub>2</sub>) to adjust medium to pH 7.2 under the N<sub>2</sub>-CO<sub>2</sub> gas phase. Tube anaerobically using 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Autoclave at 121C for 15 minutes.

*Mineral Solution I:*

K <sub>2</sub> HPO <sub>4</sub> .....	3.0 g
Distilled water.....	1.0 L

*Mineral Solution II:*

NaCl.....	12.0 g
KH <sub>2</sub> PO <sub>4</sub> .....	6.0 g
CaCl <sub>2</sub> .....	1.2 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	2.0 g
Sodium citrate.....	20.0 g
Distilled water.....	1.0 L

*VFA Solution:*

Acetic acid.....	178.33 ml
Propionic acid.....	59.60 ml
N-Butyric acid.....	38.36 ml
Isobutyric acid.....	9.50 ml
N-Valeric acid.....	9.39 ml
Isovaleric acid.....	9.31 ml
DL alpha-Methylbutyric acid.....	4.41 ml
Distilled water to.....	1.0 L

Add fatty acids to approximately 500 ml distilled water. Quickly adjust to pH 7.5 with NaOH pellets. Bring final volume to 1.0 L with additional water.

*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

*Trace Elements Solution SL-6:*

ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.10 g
MnCl <sub>2</sub> . 4H <sub>2</sub> O .....	0.03 g
H <sub>3</sub> BO <sub>3</sub> .....	0.3 g
CoCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.2 g
CuCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.01 g
NiCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.02 g
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	0.03 g
Distilled water.....	1.0 L

Adjust final pH of Trace Elements Solution to 3.4.