

ATCC medium: 1551 *Methanomicrobium* medium

Rumen fluid (clarified).....	300.0 ml
K ₂ HPO ₄	0.3 g
KH ₂ PO ₄	0.3 g
(NH ₄) ₂ SO ₄	0.3 g
NaCl.....	0.6 g
MgSO ₄ . 7H ₂ O	0.13 g
CaCl ₂ . 2H ₂ O	8.0 mg
Wolfe's Mineral Solution (see below)....	10.0 ml
Wolfe's Vitamin Solution (see below)....	10.0 ml
FeSO ₄ . 7H ₂ O	2.0 mg
NaHCO ₃	2.0 g
Yeast Extract (BD 212750).....	1.0 g
Trypticase Peptone (BD 211921).....	1.0 g
Fatty Acid Mixture (see below).....	20.0 ml
Resazurin (0.1%).....	1.0 ml
L-Cysteine . HCl.....	0.5 g
Na ₂ S . 9H ₂ O	0.5 g
Distilled water.....	660.0 ml

Adjust final pH to 6.5. Tube under 80% N₂, 20% CO₂ in aluminum seal tubes.

Fatty Acid Mixture:

Valeric acid.....	0.7 ml
Isovaleric acid.....	0.7 ml
alpha-Methylbutyric acid.....	0.5 ml
Isobutyric acid.....	0.5 ml
Distilled water.....	1.0 L

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 ₄ . 7H ₂ O	3.0 g
MnSO ₄ . H ₂ O	0.5 g
NaCl.....	1.0 g
FeSO ₄ . 7H ₂ O	0.1 g
CoCl ₂ . 6H ₂ O	0.1 g
CaCl ₂	0.1 g
ZnSO ₄ . 7H ₂ O	0.1 g
CuSO ₄ . 5H ₂ O	0.01 g
AlK(SO ₄) ₂ . 12H ₂ O.....	0.01 g
H ₃ BO ₃	0.01 g
Na ₂ MoO ₄ . 2H ₂ 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.