

**ATCC medium: 1518 *Methanlobus* medium**

KCl.....	0.335 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	2.75 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	3.45 g
NH <sub>4</sub> Cl .....	0.25 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.14 g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14 g
NaCl.....	18.0 g
Wolfe's Mineral Solution (see below)....	10.0 ml
Wolfe's Vitamin Solution (see below)....	10.0 ml
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> . 6H <sub>2</sub> O .....	2.0 mg
NaHCO <sub>3</sub> .....	5.0 g
Resazurin.....	1.0 mg
L-Cysteine . HCl.....	0.5 g
Na <sub>2</sub> S . 9H <sub>2</sub> O .....	0.5 g
Methanol (filter-sterilized).....	5.0 ml
Distilled water.....	1.0 L

Prepare medium anaerobically under 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Autoclave basal medium at 121C for fifteen minutes. Sterilize methanol separately, then add to medium at a final concentration of 0.5% (v/v). Adjust pH to 6.5.

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

*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