

ATCC medium: 1530 *Thermophilic* maintenance medium

ATCC Medium 1019 (see below) with pH adjusted to 5.6
For chemolithotrophic growth, omit fructose.

ATCC Medium 1019:

Yeast extract.....	1.0 g
NH ₄ Cl.....	1.0 g
MgSO ₄ . 7H ₂ O.....	0.1 g
KH ₂ PO ₄	0.4 g
K ₂ HPO ₄	0.4 g
Wolfe's Vitamin Solution (see below).....	10.0 ml
Wolfe's Mineral Solution (see below).....	10.0 ml
Fructose (25 ml of a 20% solution).....	5.0 g
Resazurin solution (0.01%).....	1.0 ml
Reducing Agent (see below).....	40.0 ml
NaHCO ₃	3.0 g
Distilled water.....	955.0 ml

Mix all of the ingredients except the fructose. Bring to boil to drive oxygen out. Add Reducing Agent and autoclave for 15 minutes at 121C. After autoclaving and while the medium is still hot, bubble it with oxygen-free, 90% N₂ and 10% CO₂ to maintain anaerobiosis. When the medium has cooled to about 50C, add the filter-sterilized fructose. Adjust pH if necessary to 6.7; tube aseptically under anaerobic conditions and cap with butyl rubber stoppers.

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.

Reducing Agent:

L-Cysteine . HCl.....	1.25 g
Na ₂ S . 9H ₂ O	1.25 g
Distilled water.....	100.0 ml

Boil water and cool under 100% N₂. Add cysteine, then sodium sulfide. Tube under same gas phase and autoclave at 121C for 15 minutes.