

ATCC medium: 2071 *Thermosyntropha lipolytica* medium

K ₂ HPO ₄	0.3 g
KCl.....	0.3 g
NaCl.....	0.5 g
NH ₄ Cl	1.0 g
MgCl ₂ . 6H ₂ O	0.3 g
CaCl ₂ . 2H ₂ O	50.0 mg
Olive oil.....	20.0 ml
Yeast extract.....	5.0 g
Modified Wolfe's Minerals (see below)...	10.0 ml
Wolfe's Vitamin Solution (see below)....	10.0 ml
Solution A (see below).....	50.0 ml
Solution B (see below).....	50.0 ml
Solution C (see below).....	10.0 ml
Solution D (see below).....	20.0 ml
Distilled water.....	830.0 ml

Mix ingredients through vitamin solution in 830 ml distilled water. Autoclave at 121C for 15 minutes. Cool under an atmosphere of 80% N₂, 20% CO₂. Aseptically add solutions A through D. Adjust, if necessary, to pH 9.0. Dispense aseptically using anaerobic techniques.

Modified Wolfe's Minerals:

Na ₂ SeO ₃	10.0 mg
NiCl ₂ . 6H ₂ O	10.0 mg
Na ₂ WO ₄ . 2H ₂ O.....	10.0 mg
Wolfe's Mineral Solution (see below).....	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.

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

Solution A:

NaHCO₃.....3.0 g
Distilled water.....50.0 ml

Filter-Sterilize and saturate with 100% CO₂.

Solution B:

Na₂CO₃3.0 g
Distilled water.....50.0 ml

Autoclave at 121C for 15 minutes under 100% N₂.

Solution C:

L-Cysteine . HCl.....0.15 g
Distilled water.....10.0 ml

Autoclave at 121C for 15 minutes under 100% N₂.

Solution D:

Na₂S . H₂O0.5 g
Distilled water.....20.0 ml

Autoclave at 121C for 15 minutes under 100% N₂.