

ATCC medium: 1958 *Desulfuromonas* medium

Sodium acetate . 3H ₂ O	1.36 g
Ferric citrate.....	13.7 g
NaHCO ₃	2.5 g
NaCl.....	20.0 g
KCl.....	0.67 g
Salt Solution A (see below).....	20.0 ml
Wolfe's Vitamin Solution (see below)....	10.0 ml
Modified Wolfe's Minerals (see below)....	10.0 ml
Salt Solution B (see below).....	50.0 ml
Distilled water.....	910.0 ml

Add sodium acetate and ferric citrate to distilled water. Heat to boiling to dissolve citrate, cool and adjust to pH 6.0 with NaOH. Add remaining ingredients except Salt Solution B and equilibrate under 80% N₂, 20% CO₂. Autoclave anaerobically under same gas phase at 121C for 15 minutes. Cool medium to room temperature and aseptically add Salt Solution B. Final pH of medium should be 6.9 +/- 0.1.

Salt Solution A:

NaCl.....	4.0 g
NH ₄ Cl	5.0 g
KCl.....	0.5 g
KH ₂ PO ₄	0.5 g
MgSO ₄ . 7H ₂ O	1.0 g
CaCl ₂ . 2H ₂ O	0.1 g
Distilled water.....	100.0 ml

Equilibrate Salt Solution B under 100% N₂ and autoclave under the same gas phase at 121C for 15 minutes.

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

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.

Salt Solution B:

MgCl ₂ . 6H ₂ O	21.2 g
CaCl ₂ . 2H ₂ O	3.04 g
Distilled water.....	100.0 ml