

ATCC medium: 2072 Anaerobic alkaline milk medium

KH ₂ PO ₄	0.64 g
Na ₂ HPO ₄	1.65 g
KCl.....	1.0 g
(NH ₄) ₂ SO ₄	0.5 g
NH ₄ Cl	0.5 g
MgCl ₂ . 6H ₂ O	0.4 g
CaCl ₂ . 2H ₂ O	0.4 g
Yeast extract.....	3.0 g
Skim milk.....	2.0 g
Wolfe's Vitamin Solution (see below).....	0.5 ml
Modified Wolfe's Minerals (see below)...	10.0 ml
L-Cysteine . HCl.....	0.13 g
Na ₂ S . 9H ₂ O	0.13 g
Distilled water.....	1.0 L

Adjust medium for final pH 8.0. Boil medium and cool under an atmosphere of 100% N₂. Dispense medium anaerobically under same gas phase. Autoclave 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.