

ATCC medium: 2086 Modified MS medium

Sodium sulfate.....	1.42 g
Sodium lactate syrup (85%).....	2.12 g
Yeast extract.....	2.0 g
Trypticase Peptone (BD 211921).....	2.0 g
NH ₄ Cl	1.0 g
MgCl ₂ . 6H ₂ O	1.0 g
K ₂ HPO ₄ . 3H ₂ O.....	0.4 g
CaCl ₂ . 2H ₂ O	80.0 mg
Modified Wolfe's Minerals (see below)...	10.0 ml
NaHCO ₃	4.0 g
2-Mercaptoethanesulfonic acid.....	0.2 g
Na ₂ S . 9H ₂ O	0.25 g
Distilled water.....	1.0 L

Mix ingredients through minerals in distilled water. Boil and cool under an atmosphere of 80% N₂, 20% CO₂. Add sodium bicarbonate and 2-mercaptoethanesulfonic acid. Adjust medium to pH 7.2. Dispense medium anaerobically under the same gas phase. Autoclave at 121C for 15 minutes. Just prior to inoculation, add sodium sulfide aseptically from an anaerobic, sterile stock solution to the final concentration indicated.

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.