

ATCC medium: 1871 *Acetohalobium* medium

NaCl.....	150.0 g
MgCl ₂ . 6H ₂ O	4.0 g
KCl.....	0.33 g
NH ₄ Cl	0.33 g
KH ₂ PO ₄	0.33 g
CaCl ₂ . 2H ₂ O	0.33 g
Wolfe's Mineral Solution (see below)....	10.0 ml
Wolfe's Vitamin Solution (see below)....	10.0 ml
Yeast extract.....	0.05 g
Trimethylamine . HCl *.....	2.4 g
Resazurin.....	1.0 mg
NaHCO ₃	4.0 g
Na ₂ S . 9H ₂ O	0.5 g
Distilled water.....	1.0 L

Add all components except sodium bicarbonate and sodium sulfide to distilled water. Bring to boiling and cool in an atmosphere of 80% N₂, 20% CO₂. Add sodium bicarbonate and equilibrate under same gas phase for 5 minutes. Add sodium sulfide and allow medium to reduce. Adjust medium, if necessary, for final pH 7.8 +/- 0.2 with sodium carbonate. Tube anaerobically under same gas phase. Autoclave at 121C for 15 minutes.

*Glycinebetaine may be substituted for trimethylamine at a concentration of 4.0 g/L.

Wolfe's Vitamins:

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Folic acid.....	2.0 mg
Pyridoxine hydrochloride....	10.0 mg
Riboflavin.....	5.0 mg
Biotin.....	2.0 mg
Thiamine.....	5.0 mg
Nicotinic acid.....	5.0 mg
Pantothenic acid.....	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.