

ATCC medium: 2199 *Hippea maritima* medium

NH ₄ Cl	0.33 g
CaCl ₂ . 2H ₂ O	0.33 g
MgCl ₂ . 6H ₂ O	0.33 g
KCl	0.33 g
KH ₂ PO ₄	0.33 g
NaCl	25.0 g
MOPS	3.0 g
Wolfe's Mineral Solution (see below)	10.0 ml
Wolfe's Vitamin Solution (see below)	10.0 ml
Yeast extract	0.1 g
Sodium acetate	5.0 g
Sulfur (powder)	10.0 g
Resazurin	0.5 mg
Na ₂ S . 9H ₂ O	0.5 g
Distilled water	980.0 ml

Dissolve ingredients (except vitamins, sulfur, and sulfide) in distilled water. Adjust pH to 6.0. Bring to a boil and heat under 80% N₂, 20% CO₂ for 30 minutes. Add sulfide and readjust pH to 6.0-6.2. Allow the medium to cool under gas and add vitamin solution. Dispense under the same gas into culture tubes, each containing about 0.1 g of powdered sulfur. Autoclave tubes at 110C for 20 minutes.

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