

ATCC medium: 1879 *Haloanaerobium Salsugo* medium

NaCl.....90.0 g
Yeast extract.....5.0 g
Casamino acids.....5.0 g
Mineral Solution (see below).....20.0 ml
Modified Wolfe's Minerals (see below).....5.0 ml
Wolfe's Vitamin Solution (see below).....10.0 ml
Resazurin.....1.0 mg
PIPES, dipotassium salt.....1.5 g
Cysteine-Sulfide Reducing Solution (see below).....20.0 ml
Glucose*.....10.0 g
Purified agar (if necessary).....20.0 g
Distilled water to.....1.0 L

*Add aseptically after sterilization of medium.

Adjust medium without reducing solution or glucose for final pH 6.0-7.0. Boil under 100% N₂ and cool to room temperature under the same gas phase. Add reducing solution. Dispense as required under same gas phase using anaerobic techniques. Autoclave at 121C for 15 minutes. Aseptically add sterile glucose prepared as a 20% solution under N₂ and autoclaved to yield a final concentration of 1% (i.e., 50 ml glucose per liter of medium).

Mineral Solution:

NaCl.....40.0 g
NH₄Cl.....50.0 g
KCl.....5.0 g
KH₂PO₄.....5.0 g
MgSO₄ . 7H₂O.....10.0 g
CaCl₂ . 2H₂O.....2.0 g
Distilled water to.....1.0 L

Modified Wolfe's Minerals:

Na₂SeO₃.....10.0 mg
NiCl₂ . 6H₂O.....10.0 mg
Na₂WO₄ . 2H₂O.....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
MgSO₄ . 7H₂O3.0 g
MnSO₄ . H₂O0.5 g
NaCl.....1.0 g
FeSO₄ . 7H₂O0.1 g
CoCl₂ . 6H₂O0.1 g
CaCl₂0.1 g
ZnSO₄ . 7H₂O0.1 g
CuSO₄ . 5H₂O0.01 g
AlK(SO₄)₂ . 12H₂O.....0.01 g
H₃BO₃0.01 g
Na₂MoO₄ . 2H₂O.....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

Cysteine-Sulfide Reducing Solution:

NaOH.....1.25 g
L-Cysteine . HCl.....5.0 g
Na₂S . 9H₂O5.0 g
Distilled water.....200.0 ml

Dissolve NaOH in water and boil under an N₂ gas phase. Under the same gas phase, cool the solution to room temperature, and add cysteine and sodium sulfide. Dispense anaerobically and autoclave at 121C for 15 minutes.