

ATCC medium: 1848 Carbonate buffered medium CMB4 with glucose

NaCl.....4.0 g
MgCl₂ . 6H₂O0.8 g
KCl.....0.5 g
NH₄CL0.3 g
KH₂PO₄0.2 g
Modified Wolfe's Minerals (see below)...10.0 ml
Wolfe's Vitamin Solution (see below)....10.0 ml
Resazurin.....1.0 mg
Glucose.....1.0 g
NaHCO₃2.5 g
Sulfide-Calcium Solution (see below).....2.0 ml
Distilled water.....1.0 L

Boil medium minus sodium bicarbonate and sulfide-calcium solution and cool under 80% N₂, 20% CO₂. Add sodium bicarbonate and equilibrate under same gas phase. Tube anaerobically under same gas phase. Autoclave at 121C for 15 minutes. Add sulfide-calcium solution in proportional amounts to tubed aliquots at time of inoculation. Adjust final pH to 6.9.

Sulfide-Calcium Solution:

Na₂S . 9H₂O36.0 g
CaCl₂ . 2H₂O15.0 g
Distilled water.....1.0 L

Adjust final pH to 7.2.

Equilibrate solution under 100% N₂; tube anaerobically under same gas phase. Autoclave at 121C for 15 minutes.

Modified Wolfe's Minerals:

Na₂SeO₃10.0 mg
NiCl₂ . 6H₂O10.0 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
CaCl₂.....0.1 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 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