

ATCC medium: 2260 Freshwater medium for *Geobacter*

NH ₄ Cl	0.25 g
NaH ₂ PO ₄	0.06 g
KCl.....	0.1 g
Wolfe's Vitamin Solution (see below)....	10.0 ml
Wolfe's Mineral Solution (see below)....	10.0 ml
NaHCO ₃	2.5 g
Distilled water.....	980.0 mL

Degas under 80% N₂, 20% CO₂. Autoclave at 121C for 15 minutes.

To the autoclaved medium, add the following compounds to achieve the final concentration noted:

FeNTA (Electron Acceptor) (see below), 10 mM
Sodium acetate (Electron Donor) (see below), 10 mM

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

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.

FeNTA Solution:

NaHCO₃1.64 g
Trisodium nitrilotriacetic acid....2.56 g
FeCl₃ . 6H₂O2.7 g
Distilled water to.....100.0 ml

Dissolve NaHCO₃ in 80 ml of distilled water in a 100-ml flask. Add trisodium nitrilotriacetic acid; add FeCl₃; bring volume to 100 ml; filter-sterilize using a prefilter. Do not autoclave. This is a 100 mM solution. Add approximately 1 ml of solution to 9 ml of medium to achieve 10 mM.

Sodium Acetate Solution:

Use a 100-200 mM solution of sodium acetate as electron donor. Add enough to achieve a final concentration of 10 mM.