

ATCC medium: 2557 Fresh Water Medium for Geosinus fermentans

NaHCO₃, 2.50 g
NH₄Cl, 0.25 g
NaH₂PO₄, 0.06 g
KCl, 0.10 g
Wolfe's Mineral solution (see below), 10.0 ml
Wolfe's Vitamin solution (see below), 10.0 ml
Distilled water, 800 ml

After all the ingredients are added bring the final volume up to 1 liter with distilled water. The pH should be 6.8 to 7.0. Dispense the medium (10 ml) anaerobically using 80:20 N₂:CO₂ into pressure resistant test tubes. Continue gassing out the tubes for several minutes after dispensing. Autoclave the medium at 121°C for 20 minutes. After autoclaving allow the medium to cool. Add 0.10 ml of Cysteine-HCL (3% stock solution anoxic) and exchange the headspace with 80:20 N₂:CO₂ or 80:20 H₂:CO₂. This strain seems to grow better with H₂:CO₂ gas mixture. Just before inoculation, add 0.10 ml of glucose (stock solution 2.0 M) and 0.10 ml of acetate (stock solution 2.0 M). This will give a final concentration of 20mM glucose and 20mM acetate.

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

EDTA, 0.5 g
MgSO₄·7H₂O, 3.0 g
MnSO₄·H₂O, 0.5 g
NaCl, 1.0 g
FeSO₄·7H₂O, 0.1 g
CoCl₂·6H₂O, 0.1 g
CaCl₂, 0.1 g
ZnSO₄·7H₂O, 0.1 g
CuSO₄·5H₂O, 0.01 g
AlK(SO₄)₂·12H₂O, 0.01 g
H₃BO₃, 0.01 g
Na₂MoO₄·2H₂O, 0.01 g
Na₂SeO₃ (anhydrous), 0.001 g
Na₂WO₄·2H₂O, 0.010 g
NiCl₂·6H₂O, 0.020 g
Distilled water, 1.0 L

Add EDTA 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

