

579: GEOBACTER MEDIUM

Final pH: 6.7 - 7.0

Final volume: 1011 ml

Ferric citrate (Aldrich F6129)	13.70	g
NH ₄ Cl	1.50	g
NaH ₂ PO ₄	0.60	g
KCl	0.10	g
Na-acetate	2.50	g
Modified Wolin's mineral solution	10.00	ml
NaHCO ₃	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. First dissolve ferric citrate in water by heating and adjust to pH 6.0, then add other medium ingredients except bicarbonate and vitamins. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then add and dissolve bicarbonate and adjust pH to 6.8. Thereafter, dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins to the sterile medium from an anoxic stock solution prepared under 100% N₂ gas and sterilized by filtration. Adjust pH of complete medium to 6.7 - 7.0, if necessary.

2. Note: Use 5 - 10% (v/v) inoculum.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml