579: GEOBACTER MEDIUM

Ferric citrate monohydrate 13.70 g
NH₄Cl 1.50 g
NaH₂PO₄ 0.60 g
KCl 0.10 g
Na-acetate 2.50 g
**Modified Wolin’s mineral solution** 10.00 ml
Na₂WO₄ x 2 H₂O (0.1% w/v) 0.25 ml
NaHCO₃ 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
- MgSO₄ x 7 H₂O 3.00 g
- MnSO₄ x H₂O 0.50 g
- NaCl 1.00 g
- FeSO₄ x 7 H₂O 0.10 g
- CoSO₄ x 7 H₂O 0.18 g
- CaCl₂ x 2 H₂O 0.10 g
- ZnSO₄ x 7 H₂O 0.18 g
- CuSO₄ x 5 H₂O 0.01 g
- AlK(SO₄)₂ x 12 H₂O 0.02 g
- H₃BO₃ 0.01 g
- Na₂MoO₄ x 2 H₂O 0.01 g
- NiCl₂ x 6 H₂O 0.03 g
- Na₂SeO₃ x 5 H₂O 0.30 mg
- Na₂WO₄ x 2 H₂O 0.40 mg
- Distilled water 1000.00 ml

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)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Biotin</td>
<td>20.00 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>20.00 mg</td>
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<tr>
<td>Pyridoxine hydrochloride</td>
<td>100.00 mg</td>
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<tr>
<td>Thiamine HCl</td>
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<tr>
<td>Riboflavin</td>
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<tr>
<td>Nicotinic acid</td>
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<tr>
<td>Calcium D-(+)-pantothenate</td>
<td>50.00 mg</td>
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<tr>
<td>Vitamin B$_{12}$</td>
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<tr>
<td>p-Aminobenzoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>(DL)-alpha-Lipoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
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</tbody>
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