935a: DEFERRIBACTER DESULFURICANS MEDIUM

NH₄Cl  0.33 g
KCl    0.33 g
CaCl₂ x 2 H₂O 0.33 g
MgCl₂ x 6 H₂O 0.33 g
KH₂PO₄ 0.33 g
NaCl   25.00 g
Modified Wolin’s mineral solution 10.00 ml
KNO₃   0.50 g
Na-acetate 2.00 g
Yeast extract 0.15 g
NaHCO₃ 0.30 g
Wolin’s vitamin solution (10x) 1.00 ml
Distilled water 1000.00 ml

Dissolve ingredients (except bicarbonate and vitamins), then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Adjust pH to 7.0 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins from an anoxic stock solution prepared under 100% N₂ gas and sterilized by filtration and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. The pH of the complete medium should be 7.0.

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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</thead>
<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>
</tr>
<tr>
<td>Thiamine HCl</td>
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<tr>
<td>Riboflavin</td>
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<tr>
<td>Nicotinic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Calcium D-(+)-pantothenate</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1.00 mg</td>
</tr>
<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>
</tr>
</tbody>
</table>