1577: SPOROHALOBACTER SALINUS MEDIUM

NH₄Cl 1.00 g
K₂HPO₄ 0.50 g
CaCl₂ x 2 H₂O 0.10 g
KCl 0.80 g
Na-acetate 0.50 g
NaCl 200.00 g
Yeast extract (OXOID) 0.50 g
Tryptone (BD Bacto) 5.00 g

**Modified Wolin's mineral solution** 10.00 ml
Sodium resazurin (0.1% w/v) 0.50 ml
L-Cysteine HCl x H₂O 0.50 g
MgSO₄ 5.00 g
Na₂CO₃ 1.00 g
D-Glucose 4.00 g
Na₂S x 9 H₂O 0.50 g
Distilled water 1000.00 ml

1. Dissolve ingredients (except cysteine, magnesium sulfate, carbonate, glucose and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve cysteine, adjust pH to 6.0, dispense under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add magnesium sulfate, glucose and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Prior to inoculation check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. Note: This medium cannot be stored over a longer period and should be prepared freshly before use!

**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
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<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiCl₂ x 6 H₂O</td>
<td>0.03</td>
<td>g</td>
</tr>
<tr>
<td>Na₂SeO₃ x 5 H₂O</td>
<td>0.30</td>
<td>mg</td>
</tr>
<tr>
<td>Na₂WO₄ x 2 H₂O</td>
<td>0.40</td>
<td>mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00</td>
<td>ml</td>
</tr>
</tbody>
</table>

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