283: PYRODICTIUM MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>13.85 g</td>
</tr>
<tr>
<td>MgSO₄ x 7 H₂O</td>
<td>3.50 g</td>
</tr>
<tr>
<td>MgCl₂ x 6 H₂O</td>
<td>2.75 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.33 g</td>
</tr>
<tr>
<td>NaBr</td>
<td>0.05 g</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>15.00 mg</td>
</tr>
<tr>
<td>SrCl₂ x 6 H₂O (0.1% w/v)</td>
<td>7.00 ml</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>10.00 mg</td>
</tr>
<tr>
<td>Citric acid (0.1% w/v)</td>
<td>5.00 ml</td>
</tr>
<tr>
<td>KI (0.01% w/v)</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>CaCl₂ x 2 H₂O</td>
<td>0.75 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.50 g</td>
</tr>
<tr>
<td>NiCl₂ x 6 H₂O (0.1% w/v)</td>
<td>2.00 ml</td>
</tr>
<tr>
<td>Modified Wolin's mineral solution</td>
<td>10.00 ml</td>
</tr>
<tr>
<td>Sulfur (powdered)</td>
<td>30.00 g</td>
</tr>
<tr>
<td>Sodium resazurin (0.1% w/v)</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>Yeast extract (OXOID)</td>
<td>2.00 g</td>
</tr>
<tr>
<td>Na₂S x 9 H₂O</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

1. Prepare the medium without sulfur, yeast extract and sodium sulfide, adjust pH to 5.0 - 5.5 with 10 N sulfuric acid, then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% H₂ and CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. For sterilization of medium heat vessels for at least 1 hour to 90 - 100°C on each of 3 successive days. Do not autoclave! Complete the medium by adding yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere. Adjust pH of complete medium to 5.5, if necessary.

2. After inoculation pressurize the vessels to 2 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

For DSM 2709: Reduce amount of yeast extract to 0.20 g/l.

For DSM 6158, DSM 112164: Reduce amount of yeast extract to 0.50 g/l and omit citric acid.

**Modified Wolin's mineral solution** (from medium 141)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrilotriacetic acid</td>
<td>1.50 g</td>
</tr>
<tr>
<td>MgSO₄ x 7 H₂O</td>
<td>3.00 g</td>
</tr>
<tr>
<td>MnSO₄ x H₂O</td>
<td>0.50 g</td>
</tr>
</tbody>
</table>
### 283: PYRODICTIUM MEDIUM

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1.00</td>
<td>g</td>
</tr>
<tr>
<td>FeSO(_4) \times 7\ H_2\O)</td>
<td>0.10</td>
<td>g</td>
</tr>
<tr>
<td>CoSO(_4) \times 7\ H_2\O)</td>
<td>0.18</td>
<td>g</td>
</tr>
<tr>
<td>CaCl(_2) \times 2\ H_2\O)</td>
<td>0.10</td>
<td>g</td>
</tr>
<tr>
<td>ZnSO(_4) \times 7\ H_2\O)</td>
<td>0.18</td>
<td>g</td>
</tr>
<tr>
<td>CuSO(_4) \times 5\ H_2\O)</td>
<td>0.01</td>
<td>g</td>
</tr>
<tr>
<td>AlK(SO(_4))(_2) \times 12\ H_2\O)</td>
<td>0.02</td>
<td>g</td>
</tr>
<tr>
<td>H(_3)BO(_3)</td>
<td>0.01</td>
<td>g</td>
</tr>
<tr>
<td>Na(_2)MoO(_4) \times 2\ H_2\O)</td>
<td>0.01</td>
<td>g</td>
</tr>
<tr>
<td>NiCl(_2) \times 6\ H_2\O)</td>
<td>0.03</td>
<td>g</td>
</tr>
<tr>
<td>Na(_2)SeO(_3) \times 5\ H_2\O)</td>
<td>0.30</td>
<td>mg</td>
</tr>
<tr>
<td>Na(_2)WO(_4) \times 2\ H_2\O)</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.