**718: PETROTOGA MEDIUM**

KCl 0.34 g
MgCl₂ x 6 H₂O 4.00 g
MgSO₄ x 7 H₂O 3.45 g
NH₄Cl 0.25 g
CaCl₂ x 2 H₂O 0.14 g
K₂HPO₄ 0.14 g
NaCl 18.00 g

**Modified Wolin’s mineral solution**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(NH₄)₂(SO₄)₂ x 7 H₂O (0.1% w/v)</td>
<td>2.00 ml</td>
</tr>
<tr>
<td>Sodium resazurin (0.1% w/v)</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.00 g</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>5.00 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.20 g</td>
</tr>
</tbody>
</table>

**Wolin’s vitamin solution (10x)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<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>

Dissolve ingredients (except bicarbonate, glucose, yeast extract, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 6.5 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose, yeast extract, vitamins, and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins should be sterilized by filtration. Prior to inoculation adjust pH of complete medium to 6.5 - 6.7, if necessary.

For **DSM 10691**: Supplement medium with 1.00 g/l Trypticase peptone and increase amount of yeast extract to 1 g/l.

For **DSM 13781**: Supplement medium with 5.00 g/l Na₂S₂O₃ x 5 H₂O and 5.00 g/l Trypticase peptone added from anoxic stock solutions sterilized by filtration. Omit D-Glucose. Adjust amount of yeast extract to 2.00 g/l.

For **DSM 13782**: Supplement medium with 5.00 g/l Na₂S₂O₃ x 5 H₂O and 1.00 g/l Trypticase peptone added from anoxic stock solutions sterilized by filtration. Increase amount of yeast extract to 1.00 g/l.

For **DSM 14811**: Supplement medium with 2.00 g/l Na₂S₂O₃ x 5 H₂O added from a sterile anoxic stock solution sterilized by filtration.

**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>
Microorganisms

718: PETROTOGA MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1.00</td>
<td>g</td>
</tr>
<tr>
<td>FeSO₄ x 7 H₂O</td>
<td>0.10</td>
<td>g</td>
</tr>
<tr>
<td>CoSO₄ x 7 H₂O</td>
<td>0.18</td>
<td>g</td>
</tr>
<tr>
<td>CaCl₂ x 2 H₂O</td>
<td>0.10</td>
<td>g</td>
</tr>
<tr>
<td>ZnSO₄ x 7 H₂O</td>
<td>0.18</td>
<td>g</td>
</tr>
<tr>
<td>CuSO₄ x 5 H₂O</td>
<td>0.01</td>
<td>g</td>
</tr>
<tr>
<td>Al₂(SO₄)₃ x 12 H₂O</td>
<td>0.02</td>
<td>g</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.01</td>
<td>g</td>
</tr>
<tr>
<td>Na₂MoO₄ x 2 H₂O</td>
<td>0.01</td>
<td>g</td>
</tr>
<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.

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

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Quantity</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>20.00</td>
<td>mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>20.00</td>
<td>mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>100.00</td>
<td>mg</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>50.00</td>
<td>mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>50.00</td>
<td>mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>50.00</td>
<td>mg</td>
</tr>
<tr>
<td>Calcium D-(+)-pantothenate</td>
<td>50.00</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1.00</td>
<td>mg</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>50.00</td>
<td>mg</td>
</tr>
<tr>
<td>(DL)-alpha-Lipoic acid</td>
<td>50.00</td>
<td>mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00</td>
<td>ml</td>
</tr>
</tbody>
</table>