1597: METHANOSALSUM NATRONOPHILUM MEDIUM

1. Sterilize solutions A and B in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of solution A after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 - 4 days. It is best to remove precipitates by decantation before using solution A for medium preparation.

2. Combine solution A with solution B and sparge medium with 100% N₂ gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions C to J are sterilized separately under 100% N₂ gas. Vitamins and coenzyme M should be sterilized by filtration. To complete the medium appropriate amounts of solutions C to J are added to the combined sterile solutions A and B in the sequence as indicated. Final pH of the medium should be 9.5.

3. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

**Solution A**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂CO₃</td>
<td>71.25</td>
<td>g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>11.25</td>
<td>g</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.00</td>
<td>g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.75</td>
<td>g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>750.00</td>
<td>ml</td>
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</tbody>
</table>

**Solution B**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>30.00</td>
<td>g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.25</td>
<td>g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.25</td>
<td>g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>250.00</td>
<td>ml</td>
</tr>
</tbody>
</table>
Solution C
  Trace elements solution (Pfennig & Lippert, 1966) 1.00 ml

Solution D
  Selenite-tungstate solution 1.00 ml

Solution E
  NH₄Cl 0.20 g
  MgSO₄ x 7 H₂O 0.25 g
  Distilled water 5.00 ml

Solution F
  Methanol 3.00 ml

Solution G
  Yeast extract 0.02 g
  Distilled water 5.00 ml

Solution H
  2-Mercaptoethanesulfonic acid (coenzyme M) 0.15 g
  Distilled water 10.00 ml

Solution I
  Wolin's vitamin solution (10x) 1.00 ml

Solution J
  Na₂S x 9 H₂O 0.25 g
  Distilled water 10.00 ml

Trace elements solution (Pfennig & Lippert, 1966) (from medium 1369)
  EDTA 5.00 g
  FeSO₄ x 7 H₂O 2.20 g
  ZnSO₄ x 7 H₂O 0.10 g
  MnCl₂ x 4 H₂O 0.03 g
  H₃BO₃ 0.03 g
  CoCl₂ x 6 H₂O 0.20 g
  CuCl₂ x 2 H₂O 0.03 g
  NiCl₂ x 6 H₂O 0.03 g
  Na₂MoO₄ x 2 H₂O 0.03 g
Distilled water 1000.00 ml

pH 3.0-4.0

**Selenite-tungstate solution** (from medium 385)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>0.50</td>
<td>g</td>
</tr>
<tr>
<td>Na$_2$SeO$_3$ x 5 H$_2$O</td>
<td>3.00</td>
<td>mg</td>
</tr>
<tr>
<td>Na$_2$WO$_4$ x 2 H$_2$O</td>
<td>4.00</td>
<td>mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00</td>
<td>ml</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</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 B$_{12}$</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>
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</tbody>
</table>