346: DESULFALLAS MEDIUM

**Solution A**
- Na$_2$SO$_4$: 3.00 g
- KH$_2$PO$_4$: 0.20 g
- NH$_4$Cl: 0.30 g
- CaCl$_2$ x 2 H$_2$O: 0.15 g
- MgCl$_2$ x 6 H$_2$O: 0.40 g
- NaCl: 1.00 g
- **Trace element solution SL-10**: 1.00 ml
- Sodium resazurin (0.1% w/v): 0.50 ml
- Distilled water: 965.00 ml

**Solution B**
- NaHCO$_3$: 1.00 g
- Distilled water: 20.00 ml

**Solution C**
- Ethanol: 1.00 ml
- Distilled water: 10.00 ml

**Solution D**
- Na-benzoate: 0.70 g

Sparge solution A with 100% N$_2$ gas for 30 - 45 min to make it anoxic. Thereafter, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N$_2$ and 20% CO$_2$ gas atmosphere. Anoxic stock solutions C, D, E, F, and H are prepared under 100% N$_2$ gas atmosphere and solution G under 80% N$_2$ and 20% CO$_2$ gas atmosphere. Filter sterilize solutions D, F and G. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 7.2 - 7.5, if necessary.
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Distilled water 10.00 ml

**Solution E**

**Clarified rumen fluid** 1.00 ml

**Solution F**

**Wolin's vitamin solution (10x)** 1.00 ml

**Solution G**

**Na-dithionite solution (5% w/v)** 1.00 ml

**Solution H**

Na$_2$S x 9 H$_2$O 50.00 mg
Distilled water 1.70 ml

**Trace element solution SL-10** (from medium 320)

<table>
<thead>
<tr>
<th>Salt</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl (25%)</td>
<td>10.00</td>
<td>ml</td>
</tr>
<tr>
<td>FeCl$_2$ x 4 H$_2$O</td>
<td>1.50</td>
<td>g</td>
</tr>
<tr>
<td>ZnCl$_2$</td>
<td>70.00</td>
<td>mg</td>
</tr>
<tr>
<td>MnCl$_2$ x 4 H$_2$O</td>
<td>100.00</td>
<td>mg</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>6.00</td>
<td>mg</td>
</tr>
<tr>
<td>CoCl$_2$ x 6 H$_2$O</td>
<td>190.00</td>
<td>mg</td>
</tr>
<tr>
<td>CuCl$_2$ x 2 H$_2$O</td>
<td>2.00</td>
<td>mg</td>
</tr>
<tr>
<td>NiCl$_2$ x 6 H$_2$O</td>
<td>24.00</td>
<td>mg</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$ x 2 H$_2$O</td>
<td>36.00</td>
<td>mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>990.00</td>
<td>ml</td>
</tr>
</tbody>
</table>

First dissolve FeCl$_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

**Clarified rumen fluid** (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N$_2$ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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

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

**Na-dithionite solution (5% w/v) (from medium 829)**
- NaHCO₃ 50.00 g
- Na₂S₂O₄ 50.00 g
- Distilled water 1000.00 ml

Dissolve NaHCO₃ in water and make the solution anoxic by sparging with 80% N₂ and 20% CO₂ gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.