**395a: BROCKIA MEDIUM**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>0.33 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.33 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.33 g</td>
</tr>
<tr>
<td>CaCl₂ x 2 H₂O</td>
<td>0.44 g</td>
</tr>
<tr>
<td>MgCl₂ x 6 H₂O</td>
<td>0.70 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Trace element solution SL-10</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>Sodium resazurin (0.1% w/v)</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>Sulfur (powdered)</td>
<td>10.00 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.80 g</td>
</tr>
<tr>
<td>Wolin’s vitamin solution (10x)</td>
<td>1.00 ml</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>

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4, and dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins (sterilized by filtration) and sulfide are added to the medium from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 6.5, if necessary.

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl (25%)</td>
<td>10.00 ml</td>
</tr>
<tr>
<td>FeCl₂ x 4 H₂O</td>
<td>1.50 g</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>70.00 mg</td>
</tr>
<tr>
<td>MnCl₂ x 4 H₂O</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>6.00 mg</td>
</tr>
<tr>
<td>CoCl₂ x 6 H₂O</td>
<td>190.00 mg</td>
</tr>
<tr>
<td>CuCl₂ x 2 H₂O</td>
<td>2.00 mg</td>
</tr>
<tr>
<td>NiCl₂ x 6 H₂O</td>
<td>24.00 mg</td>
</tr>
<tr>
<td>Na₂MoO₄ x 2 H₂O</td>
<td>36.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>990.00 ml</td>
</tr>
</tbody>
</table>

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>20.00 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>20.00 mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Calcium D-(+)-pantothenate</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1.00 mg</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>(DL)-alpha-Lipoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

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