### 1599: CONTUBERNALIS MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>0.50</td>
<td>g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.20</td>
<td>g</td>
</tr>
<tr>
<td>MgCl₂ x 6 H₂O</td>
<td>0.10</td>
<td>g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.20</td>
<td>g</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.20</td>
<td>g</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>3.00</td>
<td>g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.20</td>
<td>g</td>
</tr>
<tr>
<td><strong>Trace element solution SL-10</strong></td>
<td>1.00</td>
<td>ml</td>
</tr>
<tr>
<td><strong>Selenite-tungstate solution</strong></td>
<td>1.00</td>
<td>ml</td>
</tr>
<tr>
<td>Sodium resazurin (0.1% w/v)</td>
<td>0.50</td>
<td>ml</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>3.30</td>
<td>g</td>
</tr>
<tr>
<td>NaCO₃</td>
<td>6.50</td>
<td>g</td>
</tr>
<tr>
<td>Na-acetate</td>
<td>3.00</td>
<td>g</td>
</tr>
<tr>
<td>Na₂S x 9 H₂O</td>
<td>0.50</td>
<td>g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00</td>
<td>ml</td>
</tr>
</tbody>
</table>

Dissolve ingredients (except carbonates, acetate and sulfide) and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Then, add and dissolve bicarbonate and carbonate, adjust pH to 9.5, dispense under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add acetate and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Adjust pH of complete medium to 9.5 - 9.7, if necessary.

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

<table>
<thead>
<tr>
<th>Ingredient</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>FeCl₂ x 4 H₂O</td>
<td>1.50</td>
<td>g</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>70.00</td>
<td>mg</td>
</tr>
<tr>
<td>MnCl₂ x 4 H₂O</td>
<td>100.00</td>
<td>mg</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>6.00</td>
<td>mg</td>
</tr>
<tr>
<td>CoCl₂ x 6 H₂O</td>
<td>190.00</td>
<td>mg</td>
</tr>
<tr>
<td>CuCl₂ x 2 H₂O</td>
<td>2.00</td>
<td>mg</td>
</tr>
<tr>
<td>NiCl₂ x 6 H₂O</td>
<td>24.00</td>
<td>mg</td>
</tr>
<tr>
<td>Na₂MoO₄ x 2 H₂O</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 FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>0.50</td>
<td>g</td>
</tr>
<tr>
<td>Component</td>
<td>Amount</td>
<td>Unit</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------</td>
<td>------</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>