### 339a: WILKINS-CHALGREN ANAEROBE BROTH (N2/CO2)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>dehydrated Wilkins-Chalgren medium</td>
<td>33.00 g</td>
</tr>
<tr>
<td>Sodium resazurin (0.1% w/v)</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>L-Cysteine HCl</td>
<td>0.30 g</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>5.00 g</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>1.00 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

1. Dissolve 33.0 g/l dehydrated Wilkins-Chalgren medium (Oxoid CM0643) in distilled water and add 0.5 ml/l Na-resazurin solution (0.1% w/v), bring to the boil and cool to room temperature while sparging with 80% N₂ and 20% CO₂ gas mixture. Add L-cysteine-HCL x H₂O (0.3 g/l), dispense the medium into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving supplement medium with 5.0 g/l D-glucose added from a sterile anoxic stock solution sterilized by filtration. Adjust pH of medium to 6.8 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

2. Note: In case resazurin is not colourless addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) can be used to completely reduce the medium.

For DSM 14428: Replace D-glucose with 5.0 g/l cellobiose prepared under 100% N₂ gas atmosphere and sterilized by filtration.

For DSM 14924, DSM 22607: Adjust pH of medium after autoclaving to 7.4 using a sterile anoxic stock solution of Na₂CO₃ (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

For DSM 15176, DSM 15567: Omit D-glucose.

For DSM 15248: Adjust pH of medium to 6.0.

For DSM 23669: Supplement medium after autoclaving with 0.2 g/l dithiothreitol (DTT) added from a filter-sterilized anoxic stock solution and adjust pH of complete medium to 7.2.

For DSM 28816: Replace D-glucose with 3.2 g/l Na₂-fumarate prepared under 100% N₂ gas and sterilized by filtration. Adjust pH of complete medium to 7.2.