# M199 MEDIA WITH DIFFERENT HEMIN CONCENTRATIONS

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M199 Powder</td>
<td>6.6 g</td>
</tr>
<tr>
<td>2</td>
<td>HEPES</td>
<td>3.06 g</td>
</tr>
<tr>
<td>3</td>
<td>Folic Acid</td>
<td>0.09 g</td>
</tr>
<tr>
<td>4</td>
<td>Hemin</td>
<td>Variable</td>
</tr>
<tr>
<td>5</td>
<td>Adenine</td>
<td>1200 uL (0.1M Stock)</td>
</tr>
<tr>
<td>6</td>
<td>Pen Strep</td>
<td>6 mL</td>
</tr>
<tr>
<td>7</td>
<td>Gentamicin</td>
<td>750 uL</td>
</tr>
<tr>
<td>8</td>
<td>ELIX Water</td>
<td>Up to 500 mL</td>
</tr>
<tr>
<td></td>
<td>Total Volume</td>
<td>500 mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amount of Hemin added to NaOH solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>0.09g</td>
</tr>
<tr>
<td>75%</td>
<td>0.0675</td>
</tr>
<tr>
<td>50%</td>
<td>0.045</td>
</tr>
<tr>
<td>25%</td>
<td>0.0225</td>
</tr>
</tbody>
</table>
Adjust pH to 7.2 after dissolving the first 5 components in some ELIX water.

**PROCEDURE**

- Take an autoclaved measuring cylinder, weigh M199 powder, HEPES, Folic Acid, Adenine, add them to the cylinder and dissolve them in some ELIX water on a magnetic stirrer.
- Prepare 0.1M stock of Adenine by dissolving in HCl.
- Different amounts of hemin are dissolved in 1.4 M NaOH solution and added to the media.
- Now adjust the pH to 7.4 and take the media inside the hood.
- Add Pen Strep, Gentamicin the make the volume up to 500 mL using ELIX water.
- Step-up the filter unit which is autoclaved beforehand, The filter unit consists of a 0.22 um filter membrane and filtration occurs only by suction with a vacuum pump.
- Add some media to the filtration unit and check for leaks, if there are no leaks, start the vacuum pump and filter the media.
- Keep the filtered M199 media at room temperature for 24 hours. If there is any contamination we can see some precipitation, if not store the media in a 4 °C fridge.
- Add 15% FBS (425 mL M199 media + 75 mL FBS) just before the use of the media with the help of a syringe filter.