AfCS Solution Protocol

Reagent name: WEHI-231 growth medium 1
Reagent name abbreviation: WHGM1
Protocol ID: PS00000434
Version: 01
Volume: 1 L

Components:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Source</th>
<th>Catalog or Protocol No.</th>
<th>F.W. or Stock Conc.</th>
<th>Quantity</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI medium 1640</td>
<td>GIBCO/Invitrogen</td>
<td>11875093</td>
<td>100X</td>
<td>860 ml</td>
<td>0.86 X</td>
</tr>
<tr>
<td>Heat-inactivated fetal bovine serum (FBS)</td>
<td>HyClone</td>
<td>SH30071.02/Lot no. AMD16160</td>
<td>100%</td>
<td>100 ml</td>
<td>10%</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>GIBCO/Invitrogen</td>
<td>21985023</td>
<td>50 mM</td>
<td>1 ml</td>
<td>50 µM</td>
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<tr>
<td>L-Glutamine</td>
<td>GIBCO/Invitrogen</td>
<td>25030081</td>
<td>200 mM</td>
<td>10 ml</td>
<td>2 mM</td>
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<tr>
<td>Sodium pyruvate</td>
<td>GIBCO/Invitrogen</td>
<td>11360070</td>
<td>100 mM</td>
<td>10 ml</td>
<td>1 mM</td>
</tr>
<tr>
<td>HEPES</td>
<td>GIBCO/Invitrogen</td>
<td>15630080</td>
<td>1 M</td>
<td>20 ml</td>
<td>20 mM</td>
</tr>
</tbody>
</table>

Preparation:
1. The serum should be heat inactivated, as described in AfCS protocol PS00000035.
2. Pour RPMI medium 1640 into a 1-L graduated cylinder.
3. Add remaining components, cover, and mix by inversion.
4. Add solution to a 0.2-mm 1-L Nalgene SFCA filter unit in hood. Apply suction to filter.

Storage:
Temperature: 4 °C
Location: __________________
Aliquot size: NA
Special instructions: None

Author: Robert Hsueh
Date: 09/30/02
Approved: Paul Sternweis
Comments: Formulation kindly provided by Dr. Mike Gold.