AfCS Solution Protocol

Reagent name: RAW 264.7 growth medium 1
Reagent name abbreviation: RAWGM1
Protocol ID: PS00000510
Version: 01
Volume: 500 ml

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>Dulbecco’s Modified Eagle’s Medium (DMEM)</td>
<td>ATCC</td>
<td>30-2002</td>
<td>1X</td>
<td>435 ml</td>
<td>0.87X</td>
</tr>
<tr>
<td>Heat-inactivated fetal bovine serum (FBS)</td>
<td>Gemini Bio-Products</td>
<td>100-500 lot# A40802W</td>
<td>100%</td>
<td>50 ml</td>
<td>10%</td>
</tr>
<tr>
<td>HEPES</td>
<td>Invitrogen</td>
<td>15630080</td>
<td>1 M</td>
<td>10 ml</td>
<td>20 mM</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>Invitrogen</td>
<td>25030081</td>
<td>200 mM</td>
<td>5 ml</td>
<td>2 mM</td>
</tr>
</tbody>
</table>

Preparation:
1. The serum should be heat inactivated, as described in AfCS protocol PS00000247.
2. Pour 250 ml of DMEM into the top chamber of a 0.2-µm 1-L Nalgene SFCA filter unit in hood using the gradations on the top chamber.
3. Add the non-medium components with a pipette.
4. Bring the solution in the top chamber to volume with DMEM.
5. Cover and apply suction to filter.
6. Remove filter compartment and cap. Swirl to mix.

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

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Date: 08/20/03

Approved: Paul Sternweis

Comments: Macrophages are extremely sensitive to lipopolysaccharide (LPS) endotoxin from Gram-negative bacteria. All solutions, buffers, and media should be made with sterile, tissue-culture grade, endotoxin-tested water. To limit potential LPS contamination, use disposable sterile plastic rather than laboratory glassware.