AfCS Solution Protocol

Reagent name: RAW 264.7 growth medium with non-heat inactivated serum

Reagent name abbreviation: RAWGM (NHI)

Protocol ID: PS00000652

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>Fetal bovine serum (FBS)</td>
<td>Gemini Bio-Products</td>
<td>100-500</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. 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.
2. Add the non-medium components with a pipette.
3. Bring the solution in the top chamber to volume with DMEM.
4. Cover and apply suction to filter.
5. Remove filter compartment and cap. Swirl to mix.

Storage:

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

Author: Deirdre Brekken

Date: 05/27/04

Approved: Deirdre Brekken

Comments: Media using non-heat inactivated serum is to be used for all RAW cells cultured unless they are to be used as part of the ligand screen. 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.