AfCS Solution Protocol

**Reagent name:** Lentivirally transduced RAW 264.7 growth medium with 50 µg/ml hygromycin B and penicillin

**Reagent name abbreviation:** LVRGM50H+Pen

**Protocol ID:** PS00000635

**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>429.5 ml</td>
<td>0.86X</td>
</tr>
<tr>
<td>Fetal bovine serum (FBS), non-heat inactivated</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>
<tr>
<td>Penicillin G</td>
<td>Sigma-Aldrich</td>
<td>P3032</td>
<td>10,000 U/ml (6 mg/ml)</td>
<td>5 ml</td>
<td>100 U/ml</td>
</tr>
<tr>
<td>Hygromycin B</td>
<td>Roche</td>
<td>843555</td>
<td>50 mg/ml</td>
<td>500 µl</td>
<td>50 µg/ml</td>
</tr>
</tbody>
</table>

**Preparation:**
1. Note: serum is not heat inactivated, in contrast to RAWGM1 (PS00000510).
2. Pour 250 ml of DMEM into the top chamber of a 0.2-µm 1-L Nalgene SFCA filter unit set up in a laminar-flow hood (use gradations on chamber to estimate volume).
3. Add non-medium components with a pipette.
4. Bring solution in top chamber to final volume with DMEM.
5. Cover with supplied lid and apply suction to filter.
6. Remove filter compartment and cap bottle. Swirl to mix.

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

**Author:** Robert Rebres

**Date:** 06/22/04

**Approved:** Tamara Roach

**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.