AfCS Solution Protocol

Reagent name: Lentivirally transduced RAW 264.7 growth medium with 100 µg/ml G418 + 100 µg/ml Zeocin + penicillin

Reagent name abbreviation: LVRGM100G100Z+Pen

Protocol ID: PS00000682

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>G418</td>
<td>Invitrogen</td>
<td>11811-031</td>
<td>NA</td>
<td>50 µg</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Zeocin</td>
<td>Invitrogen</td>
<td>R250-01</td>
<td>100 mg/ml</td>
<td>500 µl</td>
<td>100 µg/ml</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>
</tbody>
</table>

Preparation:
1. Determine the amount of G418 to add based on lot activity reported on each bottle (e.g., 750 µg/mg active = 75% active, so 50 mg active = 66.6 mg total)
2. Note: serum is not heat inactivated, in contrast to RAWGM1 (PS00000510).
3. 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).
4. Add non-medium components with a pipette.
5. Bring solution in top chamber to final volume with DMEM.
6. Cover chamber with supplied lid and apply suction to filter.
7. Remove filter compartment and cap bottle. Swirl to mix.

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

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Date: 09/14/04

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.