AfCS Solution Protocol

Reagent name: Calcium chloride, low LPS
Reagent name abbreviation: CaCl₂, low LPS
Protocol ID: PS00000633
Version: 01
Volume: 1 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>HBSS-BSA-probenecid</td>
<td>None</td>
<td>PS00000589</td>
<td>NA</td>
<td>985 µl</td>
<td>NA</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>None</td>
<td>PS00000627</td>
<td>2 M</td>
<td>15 µl</td>
<td>30 mM*</td>
</tr>
</tbody>
</table>

Preparation:
1. Add components to 1-ml sterile microfuge tube.
2. Mix well by inversion.
3. Prepare solution fresh on day of experiment.
4. If desired CaCl₂ concentration is not found in table above, recalculate as follows to make 1 ml*:
   a. Desired concentration of CaCl₂ (mM) divided by 2 to determine volume (µl) of 2 M CaCl₂ (PS00000627) required.
   b. Bring volume to 1 ml with assay buffer (PS00000589).

*Note that different assays may require different stock concentrations of calcium chloride. Concentrations added will also vary based on previous exposure of the cells to ligand and EGTA. The final desired concentration will be recorded in the GUI.

Storage:
- Temperature: NA
- Location: __________________
- Aliquot size: NA
- Special instructions: Prepare solution fresh daily as needed.

Author: Mary Verghese
Date: 04/12/04
Approved: Grischa Chandy

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.