AfCS Ligand Protocol

Reagent name: Lipopolysaccharides, 4 milligram per milliliter
Reagent name abbreviation: LPS, 4 mg/ml
Protocol ID: PL00000033
Version: 01
Volume: 1.25 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>LPS</td>
<td>Sigma-Aldrich</td>
<td>L4524</td>
<td>NA</td>
<td>5 mg</td>
<td>4 mg/ml</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 1.25 ml of purified water to the vial of lyophilized LPS.
2. Allow solute to dissolve completely.
3. Prepare barcodes and label 1.5-ml Eppendorf tubes.
4. Divide 200-µl aliquots into barcoded Eppendorf tubes on ice.
5. Store at 4 °C.

Storage:
- Temperature: 4 °C
- Location: __________________
- Aliquot size: 200 µl
- Special instructions: None

Dilution for treatment of cells at 40 µg/ml:*
1. Dilute the ligand no earlier than 1 hr before use.
2. Dilute 150 µl of ligand stock in 1350 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration before use is 400 µg/ml.
3. Keep the diluted ligand on ice until ready to use. Warm the ligand solution to 37 °C in an environmental chamber immediately before use.

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Date: 09/16/02
Approved: Paul Sternweis

*Comments: For use in calcium assays, dilute the ligand in Hanks’ Balanced Salt Solution—Bovine Serum Albumin (HBSS-BSA), following the same procedure.