AfCS Ligand Protocol

Reagent name: 1-Oleoyl-2-hydroxy-sn-glycero-3-phosphate, lysophosphatidic acid (LPA), 10 millimolar

Reagent name abbreviation: LPA, 10 mM

Protocol ID: PL00000032

Version: 01

Volume: 5.45 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>Lysophosphatidic acid (LPA)</td>
<td>Avanti Polar Lipids</td>
<td>857130</td>
<td>458.51</td>
<td>25 mg</td>
<td>10 mM</td>
</tr>
<tr>
<td>Ethyl alcohol, 50%</td>
<td>None</td>
<td>PS00000355</td>
<td>50%</td>
<td>5.45 ml</td>
<td>50%</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Weigh 25 mg of LPA and put in a glass vial.
2. Add 5.45 ml of 50% ethyl alcohol solution to the vial of LPA.
3. Allow solute to dissolve completely.
4. Prepare barcodes and label amber glass vials.
5. Divide 100-µl aliquots into barcoded amber glass vials on ice.
6. Fill vials with argon gas and seal vials.
7. Freeze and store aliquots at –80 °C.

Storage:
Temperature: –80 °C
Location: __________________
Aliquot size: 100 µl
Special instructions: None

Dilution for treatment of cells at 1 µM:* 
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 50 µl of ligand stock in 450 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2-ml microfuge tube on ice. Invert repeatedly to mix. Dilute 15 µl of the first dilution in 1485 of SIMDM in a 2-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration before use is 10 µM.
4. 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: 04/22/02

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