AfCS Ligand Protocol

Reagent name: Sphingosine-1-phosphate (S1P), 1 millimolar

Reagent name abbreviation: S1P, 1 mM

Protocol ID: PL00000044

Version: 01

Volume: 2.64 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>Sphingosine-1-phosphate (S1P)</td>
<td>Avanti Polar Lipids</td>
<td>860492</td>
<td>379.74</td>
<td>1 mg</td>
<td>NA</td>
</tr>
<tr>
<td>Methyl alcohol, 95%</td>
<td>Mallinckrodt</td>
<td>PS00000356</td>
<td>95%</td>
<td>2.64 ml</td>
<td>95%</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 2.64 ml of 95% methyl alcohol solution to the vial of S1P.
2. Heat at 50 °C in a water bath, vortex, then sonicate in a water-bath sonicator to dissolve.
3. Prepare barcodes and label the amber glass vials.
4. Aliquot 100 µl into amber glass vials.
5. Dry to powder under a stream of N₂ gas.
6. Seal vials.
7. Freeze and store at –80 °C.

Note: Dried aliquots contain 100 nmol of S1P.

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

Dilution for treatment of cells at 0.3 µM:* 
1. Dilute the ligand no earlier than 1 hr before use.
2. Place the ligand stock on ice.
3. Add 333 µl of 1X PBS/4 mg/ml BSA to a vial of S1P (300 µM).
4. Vortex to dissolve.
5. Dilute 15 µl of ligand stock in 1485 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 3 µM.
6. 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.