AfCS Ligand Protocol

Reagent name: (5Z,11 alpha,13E,15S)-11,15-Dihydroxy-9-oxoprosta-5,13-dienoic acid, prostaglandin E$_2$ (PGE$_2$), 10 millimolar

Reagent name abbreviation: PGE, 10 mM

Protocol ID: PL00000039

Version: 01

Volume: 284 µl

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>Prostaglandin E$_2$ (PGE$_2$)</td>
<td>Sigma-Aldrich</td>
<td>P5640</td>
<td>352.5</td>
<td>1 mg</td>
<td>10 mM</td>
</tr>
<tr>
<td>Ethyl alcohol, 100%</td>
<td>Aaper Alcohol</td>
<td>030801</td>
<td>100%</td>
<td>284 µl</td>
<td>100%</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 284 µl of 100% ethyl alcohol directly to the vial of PGE$_2$.
2. Allow solute to dissolve completely.
3. Prepare barcodes and label 1.5-ml Eppendorf tubes.
4. Divide 20-µl aliquots into barcoded Eppendorf tubes on ice.
5. Freeze in liquid nitrogen and store aliquots at –80 °C.

Storage:
Temperature: –80 °C
Location: __________________
Aliquot size: 20 µl
Special instructions: None

Dilution for treatment of cells at 10 µM:*
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 15 µl of ligand stock in 1485 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2-ml Eppendorf tube on ice. Invert repeatedly to mix. The final concentration before use is 100 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.