AfCS Ligand Protocol

Reagent name: Estradiol, 10 millimolar
Reagent name abbreviation: EST, 10 mM
Protocol ID: PL00000263
Version: 01
Volume: 5 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>Estradiol</td>
<td>Sigma-Aldrich</td>
<td>E1024</td>
<td>272.4</td>
<td>13.6 mg</td>
<td>10 mM</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Aaper Alcohol</td>
<td>030801</td>
<td>100%</td>
<td>5 ml</td>
<td>100%</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Weigh 13.6 mg estradiol and place in a 15-ml conical tube.
2. Add 4 ml of 100% ethanol to estradiol and mix until dissolved.
3. Bring final volume to 5 ml with 100% ethanol and keep on ice.
4. Prepare bar codes and label 1.5-ml Eppendorf tubes.
5. Divide 50-µl aliquots into tubes on ice.
6. Freeze in liquid nitrogen and store aliquots at –80 °C.

Storage:
- Temperature: –80 °C
- Location: __________________
- Aliquot size: 50 µl
- Special instructions: None

Example: dilution of ligand for treatment of cells at 50 µM for dual ligand screen:*  
1. Dilute ligand no earlier than 1 hr before use.
2. Thaw ligand stock on ice.
3. Dilute 100 µl of ligand stock with 900 µl of appropriate assay medium. Pipette up and down with micropipette to mix. This yields a 20X treatment stock.
4. Dilute 500 µl of 20X treatment stock in 500 µl of desired assay medium (for treatment with ligand alone) or 500 µl of a 20X stock of another ligand in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration of estradiol before use is 500 µM.
5. Keep diluted ligand on ice. Immediately before use, warm ligand solution to 37 °C in an environmental chamber.

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Date: 11/05/03
Approved: Paul Sternweis

*Comments: For purposes of the dual ligand screen, a 20X stock of individual ligands is prepared initially. From the 20X stock, a 10X stock is prepared by mixing equal volumes with
assay medium or another 20X stock of a different ligand. Note that different assays use different assay media and may require different stock concentrations for addition of the ligand to the assay (see protocols for specific assays).