AfCS Ligand Protocol

Reagent name: (+/-)-Isoproterenol hydrochloride, 10 millimolar

Reagent name abbreviation: ISO, 10 mM

Protocol ID: PL00000265

Version: 01

Volume: 10 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>Isoproterenol</td>
<td>Sigma-Aldrich</td>
<td>I5627</td>
<td>247.7</td>
<td>24.8 mg</td>
<td>10 mM</td>
</tr>
<tr>
<td>Hydrochloric acid (HCl)</td>
<td>None</td>
<td>PS00000577</td>
<td>1 mM</td>
<td>10 ml</td>
<td>1 mM</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Weigh 24.8 mg isoproterenol and place in a 15-ml conical tube.
2. Add 9 ml HCl to isoproterenol and mix until dissolved.
3. Bring final volume to 10 ml with HCl 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 nM for dual ligand screen:* 
1. Dilute ligand no earlier than 1 hr before use.
2. Thaw ligand stock on ice.
3. Dilute 10 µl of ligand stock with 990 µl of appropriate assay medium containing 1 mM ascorbic acid in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix.
4. Dilute 10 µl of first dilution in 990 µl of appropriate assay medium containing 1 mM ascorbic acid in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix. This yields a 20X treatment stock.
5. 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 ISO before use is 500 nM.
6. 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).