AfCS Ligand Protocol

Reagent name: Recombinant murine interferon beta, 1.2 micromolar

Reagent name abbreviation: IFB, 1.2 µM

Protocol ID: PL00000088

Version: 01

Volume: 100 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>Recombinant murine interferon beta</td>
<td>Biosource International</td>
<td>PMC4024</td>
<td>19,700</td>
<td>1 x 10^5 units</td>
<td>1.2 µM*</td>
</tr>
</tbody>
</table>

*The molar concentration is calculated based on units/mg, which varies with each purchased lot.

Ligand stock preparation:
1. Thaw ligand on ice.
2. Prepare bar codes and label 0.6-ml Eppendorf tubes.
3. Divide 20-µl aliquots into tubes on ice.
4. Freeze in liquid nitrogen and store in aliquots at –80 °C.

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

Example: dilution of ligand for treatment of cells at 300 pM for dual ligand screen:†
1. Dilute ligand no earlier than 1 hr before use.
2. Thaw ligand stock on ice.
3. Dilute 5 µl of ligand stock with 995 µl of appropriate assay medium in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix. This yields a 20X treatment stock.
4. Dilute 500 µl of second dilution 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 IFB before use is 3 nM.
5. Keep diluted ligand on ice. Immediately before use, warm ligand solution to 37 °C in an environmental chamber.

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Date: 11/04/03

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).