AfCS Ligand Protocol

Reagent name: Interferon gamma, recombinant mouse, 10 micromolar

Reagent name abbreviation: IFG, 10 μM

Protocol ID: PL00000261

Version: 01

Volume: 640 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>Interferon gamma (IFN-γ)</td>
<td>R &amp; D Systems</td>
<td>485-MI-100</td>
<td>15,600</td>
<td>100 μg</td>
<td>10 μM</td>
</tr>
<tr>
<td>PBS/BSA</td>
<td>None</td>
<td>PS00000082</td>
<td>1X</td>
<td>640 μl</td>
<td>1X</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 640 μl PBS/BSA directly to vial of IFN-γ.
2. Mix thoroughly and allow solute to dissolve completely.
3. Prepare bar codes and label 0.6-ml Eppendorf tubes.
4. Divide 20-μl aliquots into 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

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 12 μl of ligand stock with 188 μl of appropriate assay medium. Pipette up and down with micropipette to mix.
4. Dilute 10 μl of first dilution in 990 μl of desired assay medium in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix. This yields a 20X treatment stock.
5. 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. Cap and invert repeatedly to mix. The final concentration of IFN-γ before use is 3 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).