AfCS Ligand Protocol

Reagent name: Recombinant human interferon-gamma (IFN gamma), 15.6 micromolar
Reagent name abbreviation: IFG, 15.6 µM
Protocol ID: PL00000029
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 human interferon-γ</td>
<td>BD Pharringen</td>
<td>554616</td>
<td>16,000</td>
<td>100 µl</td>
<td>15.6 µM</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Thaw ligand on ice.
2. Prepare barcodes and label 1.5-ml Eppendorf tubes.
3. Pipette 5-µl aliquots into pre-cooled, barcoded 1.5-ml Eppendorf tubes on ice.
4. Freeze in liquid nitrogen and store in aliquots at –80 °C.

Storage:
Temperature: –80 °C
Location: __________________
Aliquot size: 5 µl
Special instructions: None

Dilution for treatment of cells at 0.25 nM:*
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Add 495 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) to the 5 µl aliquot of IFG and invert several times to mix. Dilute 24 µl of the first dilution in 1476 µl of SIMDM in a 2-ml Eppendorf tube on ice and invert several times to mix. The final concentration before use is 2.5 nM.
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.