AfCS Ligand Protocol

Reagent name: Recombinant mouse interleukin-4, 14.3 µM
Reagent name abbreviation: I04, 14.3 µM
Protocol ID: PL00000004
Version: 01
Volume: 50 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 mouse IL-4</td>
<td>BD PharMingen</td>
<td>550067</td>
<td>14,000</td>
<td>0.01 mg</td>
<td>14.3 µM</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Thaw ligand on ice.
2. Prepare barcode 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 aliquots at –80 °C.

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

Dilution for treatment of cells at 0.34 nM:*  
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 2 µl of ligand stock in 198 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 1.5-ml Eppendorf tube on ice as the first dilution.
4. Dilute 36 µl of the first dilution in 1464 µl of SIMDM in a 1.5-ml Eppendorf tube on ice as the final dilution. The final concentration before stimulation is 3.4 nM.
5. 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: 04/09/02

Approved: Paul Sternweis

*Comments: For use in calcium assays, dilute the ligand in Hanks’ Balanced Salt Solution-Bovine Serum Albumin (HBSS-BSA), following the same procedure.