AfCS Ligand Protocol

Reagent name: Recombinant mouse MIP-3beta/CCL19, Ebl1-ligand chemokine (ELC), 10.6 micromolar

Reagent name abbreviation: ELC, 10.6 µM

Protocol ID: PL00000024

Version: 01

Volume: 250 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 ELC</td>
<td>R &amp; D Systems</td>
<td>440-M3 9,400</td>
<td>25 µg</td>
<td>10.6 µM</td>
<td></td>
</tr>
<tr>
<td>PBS/0.1% BSA</td>
<td>None</td>
<td>PS00000082 1X</td>
<td>250 µl</td>
<td>1X</td>
<td></td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 250 µl of PBS/0.1% BSA to the vial of lyophilized recombinant mouse ELC at room temperature.
2. Allow solute to dissolve completely and place the vial on ice.
3. Prepare barcodes and label 1.5-ml Eppendorf tubes.
4. Divide 20-µl aliquots into barcoded Eppendorf 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

Dilution for treatment of cells at 10 nM:*
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 14 µl of ligand stock in 1486 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration before use is 100 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.

Author: Robert Hsueh

Date: 04/23/02

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.