AfCS Ligand Protocol

Reagent name: Interleukin-6, recombinant mouse, 500 nanomolar

Reagent name abbreviation: I06, 500 nM

Protocol ID: PL00000224

Version: 01

Volume: 486 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>Interleukin-6 (IL-6)</td>
<td>Sigma-Aldrich</td>
<td>I9646</td>
<td>20,600</td>
<td>5 µg</td>
<td>500 nM</td>
</tr>
<tr>
<td>PBS/BSA</td>
<td>None</td>
<td>PS000000082</td>
<td>1X</td>
<td>486 µl</td>
<td>1X</td>
</tr>
</tbody>
</table>

Ligand stock preparation:

1. Add 486 µl PBS/BSA directly to vial containing IL-6.
2. Mix thoroughly and allow solute to dissolve completely.
3. Prepare bar codes and label 0.6-ml Eppendorf tubes.
4. Divide 10-µl aliquots into tubes on ice.
5. Freeze in liquid nitrogen and store aliquots at –80 ºC.

Storage:

Temperature: –80 ºC
Location: __________________
Aliquot size: 10 µl
Special instructions: None

Example: dilution of ligand for treatment of cells at 100 pM for dual ligand screen:

1. Dilute ligand no earlier than 1 hr before use.
2. Thaw ligand stock on ice.
3. Dilute 4 µl of ligand stock with 996 µl of appropriate assay medium. 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 20X stock of another ligand in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration of IL-6 before use is 1 nM.
5. Keep diluted ligand on ice. Immediately before use, warm ligand solution to 37 ºC in an environmental chamber.

Author: Robert Hsueh/Yan Ni/Jason Polasek

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).