AfCS Ligand Protocol

**Reagent name:** Purified hamster anti-mouse CD40 monoclonal antibody, 6.7 µM

**Reagent name abbreviation:** 40L, 6.7 µM

**Protocol ID:** PL00000001

**Version:** 01

**Volume:** 500 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>Anti-mouse CD40</td>
<td>BD PharMingen</td>
<td>553721</td>
<td>150,000</td>
<td>0.5 mg</td>
<td>6.7 µM</td>
</tr>
</tbody>
</table>

**Ligand stock preparation:**

1. Prepare barcode and label ligand.
2. Store the ligand at 4 °C.

**Storage:**

- Temperature: 4 °C
- Location: __________________
- Aliquot size: NA
- Special instructions: None

**Dilution for treatment of cells at 65 nM:**

1. Dilute the ligand no earlier than 1 hr before use.
2. Dilute 146 µl of ligand stock in 1354 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 1.5-ml Eppendorf tube on ice. The final concentration before stimulation is 652 nM.
3. 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:** Zhen Yan

**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.