AfCS Ligand Protocol

**Reagent name:** Recombinant human transforming growth factor-beta 1 (TGF-β1), 400 nanomolar

**Reagent name abbreviation:** TGF, 400 nM

**Protocol ID:** PL00000045

**Version:** 01

**Volume:** 200 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 TGF-β1</td>
<td>R &amp; D Systems</td>
<td>240-B</td>
<td>25,000</td>
<td>2 µg</td>
<td>400 nM</td>
</tr>
</tbody>
</table>

**Ligand stock preparation:**

1. Make 2ml of a 4mM HCL/0.1% BSA solution by adding 8 µl of 1M HCL and 5 µl of 40 mg/ml BSA to 1.987 ml of purified water. Cap and mix thoroughly.
2. Add 200 µl of 4 mM HCl/0.1% BSA to the vial of lyophilized TGF-β1.
3. Allow solute to dissolve completely.
4. Prepare barcodes and label 1.5-ml Eppendorf tubes.
5. Divide 20-µl aliquots into barcoded Eppendorf tubes on ice.
6. 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 400 pM:**

1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 15 µl of ligand stock in 1485 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2-ml Eppendorf tube on ice. Invert several times to mix. The final concentration before use is 4 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:** 07/03/02

**Approved:** Zhen Yan

**Comments:** Our preliminary experiments did not show positive responses in primary B cells at a concentration as high as 4 nM. Therefore, we chose this concentration based on previous findings by Patil et al. (J Biol Chem, 275[49]:38363, 2000). For use in calcium assays, dilute the
ligand in Hanks' Balanced Salt Solution—Bovine Serum Albumin (HBSS-BSA), following the same procedure.