AfCS Ligand Protocol

Reagent name: N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), 10 millimolar

Reagent name abbreviation: FML, 10 mM

Protocol ID: PL00000025

Version: 01

Volume: 1.14 ml

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>N-formyl-met-leu-phe (fMLP)</td>
<td>Sigma-Aldrich</td>
<td>F3506</td>
<td>437.6</td>
<td>5 mg</td>
<td>10 mM</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>Sigma-Aldrich</td>
<td>D2650</td>
<td>100%</td>
<td>1.14 ml</td>
<td>100%</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 1.14 ml of DMSO to the vial of fMLP 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 25-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: 25 µl
- Special instructions: None

Dilution for treatment of cells at 100 nM:*
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 10 µl of ligand stock in 990 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 1.5-ml Eppendorf tube on ice. Dilute 15 µl of the first dilution in 1485 µl of SIMDM in a 2-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration before use is 1 µM.
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: 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.