AfCS Ligand Protocol

Reagent name: Bombesin, 6.2 millimolar

Reagent name abbreviation: BOM, 6.2 mM

Protocol ID: PL00000012

Version: 01

Volume: 617 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>Bombesin</td>
<td>Sigma-Aldrich</td>
<td>B4272</td>
<td>1,620</td>
<td>1 mg</td>
<td>6.2 mM</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>None</td>
<td>PS00000357</td>
<td>50 mM</td>
<td>100 µl</td>
<td>50 mM</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Add 100 µl of acetic acid solution directly to solid bombesin in vial 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 5-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: 5 µl
Special instructions: None

Dilution for treatment of cells at 1.0 µM:* 
1. Dilute the ligand no earlier than 1 hr before use.
2. Thaw the ligand stock on ice.
3. Dilute 3 µl of ligand stock in 1857 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2.0-ml Eppendorf tube on ice. Invert repeatedly to mix. The final concentration before use is 10 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: 04/22/02

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.