AfCS Ligand Protocol

Reagent name: 2-Methylthioadenosine 5'-triphosphate tetrasodium (2-methyl-thio-ATP), 10 millimolar

Reagent name abbreviation: 2MA, 10 mM

Protocol ID: PL00000009

Version: 01

Volume: 156 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>2-Methylthioadenosine 5'-triphosphate tetrasodium (2MA)</td>
<td>Sigma-Aldrich</td>
<td>A-023</td>
<td>641.2</td>
<td>1 mg</td>
<td>10 mM</td>
</tr>
</tbody>
</table>

Ligand stock preparation:
1. Prepare ligand stock fresh, no earlier than 1 hr before each experiment.
2. Take vial of 2MA out of −80 °C freezer and warm to room temperature.
3. Weigh 1 mg of 2MA and place in a barcoded 1.5-ml Eppendorf tube.
4. Add 156 µl of purified water directly to the tube of 2MA. If the weight is not 1 mg, adjust the volume of water accordingly.
5. Allow solute to dissolve completely.
6. Store the ligand on ice until use.

Storage:
Temperature: NA
Location: __________________
Aliquot size: NA
Special instructions: None

Dilution for treatment of cells at 100 µM:*
1. Dilute the ligand no earlier than 1 hr before use.
2. Dilute 150 µl of ligand stock in 1350 µl of Supplemented Iscove’s Modified Dulbecco’s Medium (SIMDM) in a 2-ml Eppendorf tube on ice. Invert repeatedly to mix. The final concentration before use is 1 mM.
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.

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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.