AfCS Ligand Protocol

**Reagent name:** PAM\textsubscript{2}Cys-SKKKK x 3 TFA (S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-[R]-cysteiny1-[S]-seryl-[S]-lysyl-[S]-lysyl-[S]-lysyl-[S]-lysyl x 3 CF\textsubscript{3}COOH), 1 millimolar

**Reagent name abbreviation:** P2C, 1 mM

**Protocol ID:** PL00000246

**Version:** 01

**Volume:** 1 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>PAM\textsubscript{2}Cys-SKKKK x 3 TFA (P2C)</td>
<td>EMC Microcollections</td>
<td>L2020</td>
<td>1271.85</td>
<td>1 mg</td>
<td>1 mM</td>
</tr>
<tr>
<td>Distilled water, endotoxin free</td>
<td>Invitrogen</td>
<td>15230162</td>
<td>NA</td>
<td>782 µl</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Ligand stock preparation:**

1. Add 1 ml water directly to vial of P2C.
2. Mix thoroughly and allow solute to dissolve completely.
3. Prepare bar codes and label 0.6-ml Eppendorf tubes.
4. Divide 15-µl aliquots into tubes on ice.
5. Freeze in liquid nitrogen and store aliquots at –80 °C.

**Storage:**

Temperature: –80 °C
Location: ________________
Aliquot size: 15 µl
Special instructions: None

**Example: dilution of ligand for treatment of cells at 350 nM for dual ligand screen:**

1. Dilute ligand no earlier than 1 hr before use.
2. Thaw ligand stock on ice.
3. Dilute 7 µl of ligand stock with 993 µl of appropriate assay medium. Invert repeatedly to mix. This yields a 20X treatment stock.
4. Dilute 500 µl of first dilution in 500 µl of desired assay medium (for treatment with ligand alone) or 500 µl of a 20X stock of another ligand in a 1.5-ml microfuge tube on ice. Invert repeatedly to mix. The final concentration of P2C before use is 3.5 µM.
5. Keep diluted ligand on ice. Immediately before use, warm ligand solution to 37 °C in an environmental chamber.

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**Date:** 11/05/03

**Approved:** Paul Sternweis
*Comments:* For purposes of the dual ligand screen, a 20X stock of individual ligands is prepared initially. From the 20X stock, a 10X stock is prepared by mixing equal volumes with assay medium or another 20X stock of a different ligand. Note that different assays use different assay media and may require different stock concentrations for addition of the ligand to the assay (see protocols for specific assays).