AfCS Solution Protocol

Reagent name: Phosphospecific antibody mixture 4 for RAW 264.7 cells
Reagent name abbreviation: Ab mix 4R
Protocol ID: PS00000563
Version: 01
Volume: Variable, indicated in solution GUI

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>BSA, 5%/TBST, 0.1%</td>
<td>None</td>
<td>PS00000020</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Phospho-PKC(\delta/\theta) (Ser643/676)</td>
<td>Cell Signaling Technology</td>
<td>9376</td>
<td>NA</td>
<td>1:8000 dilution</td>
<td>NA</td>
</tr>
<tr>
<td>Phospho-Smad2 (Ser465/467)</td>
<td>Cell Signaling Technology</td>
<td>3101</td>
<td>NA</td>
<td>1:8000 dilution</td>
<td>NA</td>
</tr>
<tr>
<td>Phospho-p40 phox (Thr154)</td>
<td>Cell Signaling Technology</td>
<td>4311</td>
<td>NA</td>
<td>1:1000 dilution</td>
<td>NA</td>
</tr>
<tr>
<td>RhoGDI</td>
<td>BD Biosciences</td>
<td>R26320</td>
<td>NA</td>
<td>1:200,000 dilution</td>
<td>NA</td>
</tr>
</tbody>
</table>

Preparation:
1. Add 5% BSA/0.1% TBST to a 15- to 50-ml plastic tube with tight-fitting cap.
2. Add antibodies and rinse pipette tip into solution.
3. Cap tube and mix by inversion.

Storage:
Temperature: 4 °C
Location: ________________
Aliquot size: NA
Special instructions: None

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Date: 10/21/03
Approved: Susanne Mumby

Comments: Solution may be reused for at least three independent Western blotting experiments. Number of uses should be marked on tube. Both anti-rabbit and anti-mouse secondary antibodies (anti-rabbit HRP + Alexa Fluor 647 anti-mouse, PS00000564) are needed to detect all primary antibodies in this mixture.