AfCS Solution Protocol

**Reagent name:** Phosphospecific antibody mixture 1  
**Reagent name abbreviation:** Ab mix 1  
**Protocol ID:** PS00000312  
**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>5% BSA/0.1% TBST</td>
<td>None</td>
<td>PS00000020</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Monoclonal anti-MAP kinase, activated</td>
<td>Sigma-Aldrich</td>
<td>M8159</td>
<td>NA</td>
<td>1:5000 dilution</td>
<td>NA</td>
</tr>
<tr>
<td>(Diphosphorylated ERK 1 &amp; 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospho-Akt (Ser473)</td>
<td>Cell Signaling Technology</td>
<td>9271</td>
<td>NA</td>
<td>1:500 dilution</td>
<td>NA</td>
</tr>
<tr>
<td>Phospho-Stat6 (Tyr641)</td>
<td>Cell Signaling Technology</td>
<td>9361</td>
<td>NA</td>
<td>1:3000 dilution</td>
<td>NA</td>
</tr>
<tr>
<td>Phospho-p90RSK (Ser380)</td>
<td>Cell Signaling Technology</td>
<td>9341</td>
<td>NA</td>
<td>1:1000 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 phosphospecific antibodies and rinse pipette tip into the solution.  
3. Cap tube and mix by inversion.

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

**Author:** Erica Turon  
**Date:** 02/19/02  
**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 are needed to detect all primary antibodies in this mixture.