

# Phospho Antibody Stripping Solution

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**CATALOG NUMBER:** AKR-102

**STORAGE:** Room Temperature

**QUANTITY AND CONCENTRATION:** 10mL at 75X concentration

**SHELF LIFE:** 1 year from receipt under proper storage conditions; avoid multiple freeze thaw cycles

## **Background**

Protein phosphorylation-dephosphorylation is one of the major signaling mechanisms for modulating the functional properties of proteins involved in gene expression, cell adhesion, cell cycle, cell proliferation, and differentiation. Proteins can be phosphorylated by protein kinases on specific serine, threonine, or tyrosine residues. The utilization of anti-phosphoprotein antibodies in western blotting has become a commonly used tool for signal transduction research. The ability to effectively strip phospho antibody blots and re-probe provides several advantages:

- Cost savings in reagents and time by reusing the same blot.
- Analysis of a given blot using several different antibodies.
- Re-analysis of anomalous results and confirmation with the same or a different antibody.

Cell Biolabs' Phospho Antibody Stripping Solution is designed to specifically and effectively remove anti-phosphotyrosine antibodies from blots without significantly affecting the immobilized proteins.

Advantages of the Phospho Antibody Stripping Solution include:

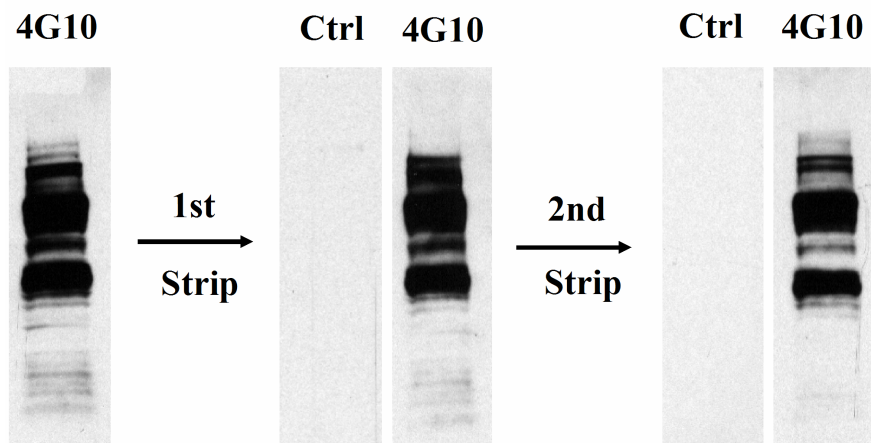
- Specially formulated for stripping anti-phosphotyrosine antibodies.
- Allows multiple strippings without significantly affecting the immobilized proteins.
- Antibody stripping is done at room temperature. No heating of blots is required.

## **Methods**

1. Freshly prepare enough 1X Phospho Antibody Stripping Solution in a vented chemical hood by diluting the 75X stock in dH<sub>2</sub>O, mix well.
2. Submerge the blot in the 1X Phospho Antibody Stripping Solution and incubate with gentle mixing for 90 to 120 minutes at room temperature. Ensure sufficient volume is used to completely cover the blot.
3. Wash twice with TBST.
4. Test for complete removal of the phospho-antibody by blotting with secondary antibody, followed by ECL detection.
5. After determining that the membrane is properly stripped, the blot is now ready for re-probing with same or different antibodies.

## **Example of results**

The following figures demonstrate typical titration results. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1. Multiple Stripping of 4G10 Phosphotyrosine Antibody.** 15  $\mu$ g of serum-stimulated COS-7 cell lysate was subject to 4G10 anti-phosphotyrosine western blot. The same blot was stripped with Phospho Antibody Stripping Solution and re-probed with either HRP-secondary conjugate alone (Ctrl) or the primary 4G10 antibody and the HRP-secondary conjugate (4G10), followed by detection with ECL reagents. Films were exposed for 30 seconds for the 4G10 blot and 30 minutes for the control blot.

### References

1. Arthur, W.T., Ellenbroek, S.M., Der, C.J., and Burrridge K. (2002) *J. Biol. Chem.* **277**, 42964-42972
2. Garcia-Mata R., Wennerberg, K., Arthur, W.T., Noren, N.K., Ellenbroek, S.M., and Burrridge K. (2006) *Methods Enzymol.* **406**, 425-437.

### Warranty

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