

Phosphotyrosine Detection Kit Cat. No. 525322

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Introduction

Protein phosphorylation is the principal indicator of the activation of signaling pathways. The binding of a growth factor to its specific receptor catalyzes a complex cascade of intracellular signaling events, characterized by altering the phosphorylation of tyrosine, serine, or threonine residues of target proteins.

The phosphorylation state is tightly regulated by the actions of protein kinases, which transfer a phosphate group from donor substrates such as ATP or GTP to serine, threonine, or tyrosine residues, and protein phosphatases, which dephosphorylate phosphorylated proteins, thereby restoring the particular phosphorylation system to its basal stage. Most kinases are either serine/threonine-specific or tyrosine-specific, while some kinases can phosphorylate all 3 types of hydroxy-bearing amino acids.

Radioactive kinase assays utilizing ^{32}P - γ -ATP as the donor substrate have been frequently employed to assess kinase activity, since the method is sensitive, convenient, and easily quantified. Recently, however, the generation of phosphoprotein-specific antibodies has advanced the development of non-radioactive kinase activity detection methods to comparable sensitivity as radioactive methods.

Background and Specificity

Antibodies recognizing phosphorylated epitopes recognize the phosphorylated amino acid in the context of the surrounding amino acid sequence. Recognition, therefore, depends on 2 criteria: 1) phosphorylation and 2) the surrounding amino acid motif. If one of the two criteria is not fulfilled, the antibody will not detect the phosphorylation site. Since the surrounding amino acid sequence varies between phosphorylation sites, certain proteins—though phosphorylated—may not be detected by a given antibody.

Therefore, Calbiochem® brand offers a set of four different monoclonal antibodies specific for tyrosine phosphorylation sites. The phosphorylation patterns in a given cell extract may differ when probed with individual antibodies due to sequence specificity. These antibodies are suitable for use in human, mouse, rat, chicken, frog, zebrafish, and yeast cell extracts. The antibodies are not suitable for use with bacterial cell lysates.

This set contains 25 μg of each of the following phosphotyrosine antibodies:

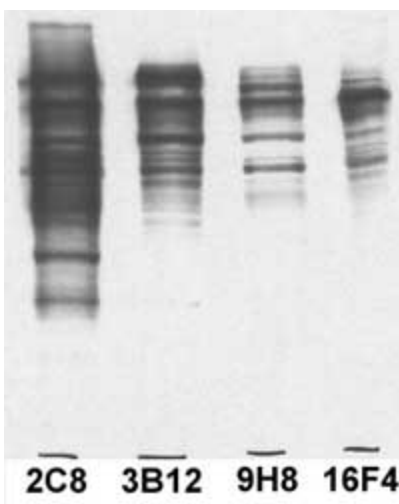
Clone Name	Host	Isotype	Immunoblotting*	Immunoprecipitation	ELISA*
2C8	Mouse	IgG ₁	0.1-1 $\mu\text{g}/\text{ml}$	1-10 $\mu\text{g}/10^6$ cells	0.05 $\mu\text{g}/\text{ml}$
3B12	Mouse	IgG ₁	0.1-1 $\mu\text{g}/\text{ml}$	1-10 $\mu\text{g}/10^6$ cells	0.05 $\mu\text{g}/\text{ml}$
9H8	Mouse	IgG ₁	0.1-1 $\mu\text{g}/\text{ml}$	1-10 $\mu\text{g}/10^6$ cells	0.05 $\mu\text{g}/\text{ml}$
16F4	Mouse	IgG ₁	0.1-1 $\mu\text{g}/\text{ml}$	1-10 $\mu\text{g}/10^6$ cells	0.05 $\mu\text{g}/\text{ml}$

Reconstitute each antibody in 1 ml of distilled water at room temperature. Aliquot and store at -70°C. *Use BSA for membrane blocking and antibody dilutions, **do not use milk/casein**.

Positive Control

The Calbiochem® Phosphotyrosine Detection Kit includes a positive control consisting of seven phosphotyrosine-modified proteins: galactosidase (114 kDa), phosphorylase A (94 kDa), conalbumin (78 kDa), BSA (67 kDa), ovalbumin (47 kDa), carbonic anhydrase (30 kDa), and soybean trypsin inhibitor (21 kDa).

Reconstitute the positive control in 500 µl of water. After solubilization, add 500 µl of SDS-PAGE sample buffer and incubate at 90°C for 5 min. Aliquot and store at -20°C. Avoid freeze/thaw cycles. For immunoblotting on PVDF membranes, apply 20 µl/lane (mini gel) for colorimetric detection or 10 µl/lane (mini gel) for chemiluminescent detection. **Do not use nitrocellulose membranes!**



Detection of phosphotyrosine-containing proteins by immunoblotting. Sample: Whole cell lysates from A431 cells treated with pervanadate (all lanes). Primary antibody: Anti-Phosphotyrosine (2C8) Mouse mAb (Cat. No. 525293) (0.5 µg/ml), Anti-Phosphotyrosine Mouse mAb (3B12) (0.5 µg/ml), Anti-Phosphotyrosine Mouse mAb (9H8) (0.5 µg/ml), and Anti-Phosphotyrosine Mouse mAb (16F4) (0.5 µg/ml). Detection: chemiluminescence.

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