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ProductInformation

Monoclonal Anti-Phosphotyrosine
FITC Conjugate
Clone PT-66
Immunoglobulin Fraction of Mouse Ascites Fluid

Product Number **F 3145**

Product Description

Monoclonal Anti-Phosphotyrosine (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A phosphotyrosine-BSA conjugate was used as the immunogen. The isotype is determined using Sigma ImmunoType™Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The immunoglobulin fraction of mouse ascites fluid is conjugated to FITC (isomer I) and then purified by gel filtration to remove free FITC

Monoclonal Anti-Phosphotyrosine is specific for phosphorylated tyrosine, both as the free amino acid or when conjugated to carriers such as BSA or KLH. It does not react with non-phosphorylated tyrosine or other phosphorylated amino acids, including serine and threonine, nor does it react with phosphorylated molecules such as AMP or ATP. In immunoblotting, the antibody has been used to localize phophotyrosine-containing proteins in a preparation of human platelets and in the cultured human epidermoid carcinoma cell line A-431 (e.g. EGF receptor after EGF stimulation). Monoclonal Anti-Phosphotyrosine has also been used for the immunofluorescent labeling of tyrosine-phosphorylated proteins at focal adhesion and cellular junctions of cultured MDCK cells.

Protein phosphorylation is a basic mechanism for the modification of protein function in eukaryotic cells. Tyrosine phosphorylation is a rare post-translational event in normal tissue, accounting for only 0.03% of phosphorylated amino acids. The level of phosphorylated tyrosine in many cellular proteins increases ten-fold following various activation processes which are mediated through phosphotyrosine kinases.

The importance of tyrosine phosphorylation has been stablished by demonstrating that it is an integral response in many different mitogenic receptor systems. For example, many of the mitogenic receptor systems such as the EGF, PDGF and insulin receptors contain tyrosine kinase domains. When the ligand binds to the receptor autophosphorylation of tyrosine residues occurs. Other receptors, T-cell antigen receptor complex or some of the hemopoietic growth factor receptors, are capable of stimulating associated tyrosine kinase. For example, the CD4 and CD8 antigens are coupled to a protein-tyrosine kinase that phosphorylates the CD3 complex. Tyrosine-specific protein kinase activity has also been described in many retroviral oncogene proteins. Cells transformed by these oncogenes contain elevated levels of phosphotyrosine. Many of the oncogenes found in mammalian oncogenic viruses encode tyrosine protein kinases that reside in the cellular cytoplasm. Others encode transmembrane receptors whose tyrosine phosphotransferase activity is stimulated by the binding of ligand to the extracellular domain. Many studies suggest that there are both common and specific substrates for viral oncogene and growth factor receptor tyrosine kinases. The role of tyrosine kinases in signal transduction pathways is evidenced by the observation that mutation, which abolish kinase activity depend on the identification of their substrate and a subsequent determination of how phosphorylation affects the properties of these proteins.

Studies on the role of phosphotyrosyl-protein have been hampered by their low concentrations and the problem of distinguishing them from phosphoseryl and phosphothreonyl proteins. The autoradiography method based on the resistance of phosphotyrosine to alkaline hydrolyses is not very sensitive because not all of the other phosphoamino acids are completely hydrolyzed, resulting in high backgrounds. Consequently, antibodies which are specific for phosphotyrosine allow for better analysis of phosphotyrosine.

Monoclonal Anti-Phosphotyrosine may be used for the identification of proteins containing phosphorylated tyrosine in phytohemagglutination (PHA)-activated human lymphocytes in flow cytometry.

Reagents
Monoclonal Anti-Phosphotyrosine FITC Conjugate is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Precautions and Disclaimer
Consult the MSDS for informaiton regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile
1. F-DIBA (Fluorescent Dot Immunobinding Assay): 1:64-1:128
   The titer of the conjugate was determined on a 5-10 microgram dot of phosphtyrosine-BSA bound to nitrocellulose.

2. P-IFMA (Particle Immunofluorescent Assay): 1:32-1:64
   The titer of the conjugate was determined using a 50 µl suspension of phosphotyrosine-BSA agarose coated with approximately 100 µg of phosphotyrosine-BSA.

In order to obtain best results it is recommended that each individual user determine their working dilution by titration assay.

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