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ProductInformation

MONOCLONAL ANTI-PHOSPHOTYROSINE
CLONE PT-66
Mouse Ascites Fluid

Product No. **P 3300**

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).

This antibody 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 nonphosphorylated tyrosine or other phosphorylated amino acids, including serine and threonine, nor does it react with phosphorylated molecules such as AMP or ATP.

In an immunoblot technique, the antibody has been used for the localization of phosphotyrosine 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.

Monoclonal Anti-Phosphotyrosine may be used for the identification of proteins containing phosphorylated tyrosine in immunohistochemisty, flow cytometry, immunoprecipitation,[5] immunoblotting,[5] ELISA, RIA or for immunoaffinity isolation.[1-5]

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 tenfold following various activation processes which are mediated through phosphotyrosine kinases.

The importance of tyrosine phosphorylation has been established by the demonstration that it is an integral response in many different mitogenic receptor systems. For instance, 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 factors 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 mutations which abolish kinase activity depends 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 phospho-amino acids are completely hydrolyzed, resulting in high backgrounds. Consequently, antibodies which are specific for phosphotyrosine allow for better analysis of phosphotyrosine.

Reagents
The product is provided as ascites fluid containing 15 mM sodium azide.

Precautions and Disclaimer
Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage and Stabilty
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. ELISA: Minimum 1:1,600
   The antibody titer was determined using phosphotyrosine bound to carrier (BSA or KLH) at 10 µg/ml as the antigen. In a competitive ELISA binding is inhibited by phosphotyrosine with no inhibition observed using nonphosphorylated tyrosine, phosphothreonine, phosphoserine or ATP.

2. Immunoblotting: Minimum 1:2,000
   The antibody titer was determined by an indirect assay staining phosphotyrosine in a fresh human platelet preparation.

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

References

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