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ProductInformation

MONOCLONAL ANTI-PHOSPHOTYROSINE
CLONE PY-20
Purified Mouse Immunoglobulin

Product Number **P 4110**

Product Description

Monoclonal Anti-Phosphotyrosine (mouse IgG2b isotype) is derived from the PY20 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A phosphotyrosine-protein conjugate was used as the immunogen.[1,2] The antibody is purified from ascites fluid using protein A chromatography.

Monoclonal Anti-Phosphotyrosine is specific for both native and denatured proteins containing phosphorylated tyrosine. Antibody binding is inhibited with phosphotyrosine and phenylphosphate, but not phosphoeserine or phosphothreonine.[1,2]

Monoclonal Anti-Phosphotyrosine may be used as an analytical tool by enabling the identification and quantification of tyrosine-phosphorylated proteins. The antibody may be used for immunoblotting, ELISA, immunoprecipitation and immunohistochemistry.

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 that 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 observed with mutations which abolish kinase activity and depends on the identification of their substrates and a subsequent determination of how phosphorylation affects the properties of these proteins.

Low concentrations and the problem of distinguishing them from phosphoseryl and phosphothreonyl proteins have hampered studies on the role of phosphotyrosyl-protein. 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 specific for phosphotyrosine allow for better analysis of phosphotyrosine.

Reagents

Monoclonal Anti-Phosphotyrosine is provided as a solution at approximately 1 mg/ml in 20 mM phosphate buffered saline, pH 7.5, containing 50% glycerol and 3 mM sodium azide.

Precautions
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
Store at –0 °C to –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
A suggested working dilution of 1:2,000 is determined for immunoblotting and ELISA. A suggested working dilution of 1:500 is determined for immunohisto-chemistry and immunocytochemistry.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

1. Glenney, J.R., et al., J. Immunol. Meth., **109**, 277-285 (1988).
2. Ruff-Jamison, AS., et al., J. Biol. Chem., **266**, 6607-6613 (1991).

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