



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-PHOSPHOTYROSINE - AGAROSE Clone PT-66

Product No. **A 1806**

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 antibody is then purified by High Performance Affinity Chromatography (Protein A column). After purification the antibody preparation is immobilized on cyanogen-bromide activated agarose.

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.

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 hemo-poietic 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 phosphoserine and phosphothreonine 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 - Agarose may be used for the immunoprecipitation or immunoaffinity purification procedures of phosphotyrosyl proteins, including transforming proteins, steroid hormone receptors (e.g., estradiol and glucocorticoid receptors), growth factor receptors (e.g., EGF and PDGF receptors), insulin receptors and other proteins involved in the control of cellular growth.

Reagents

The product is provided as a 1:1 suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

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

Product Profile

Each milliliter of settled resin will bind at least 1 mg of phosphotyrosine-BSA conjugate. A partial elution can be achieved with 40 mM phenyl phosphate.

For microscale immunoprecipitation or immuno-purification of tyrosine phosphoproteins from cell lysates we recommend determination of the optimum amount of antibody-agarose be resolved by a titration assay.

Storage

Store at 2-8 °C. **Do Not Freeze.**

Pcs1/00

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.

Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.

Please see reverse side of the invoice or packing slip.