Product Information

Monoclonal Anti-Phosphotyrosine-Peroxidase Antibody

Produced in Mouse, Clone PT-66, Purified Immunoglobulin, Lyophilized Powder

A5964

Product Description

Monoclonal Anti-Phosphotyrosine (mouse IgG1 isotype) is derived from the PT-66 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a phosphotyrosine-BSA conjugate. The immunoglobulin fraction of antibody to phosphotyrosine is purified from ascites fluid and then conjugated to horseradish peroxidase (HRP).

Monoclonal Anti-Phosphotyrosine-Peroxidase reacts specifically with phosphorylated tyrosine when conjugated to carriers such as BSA using dot blot and ELISA. It does not react with other phosphorylated amino acids, such as phosphoserine and phosphothreonine.

Protein phosphorylation is a basic mechanism for the modification of protein function in eukaryotic cells.[1-3] Tyrosine phosphorylation is a rare post-translational event in normal tissue, accounting to only 0.03% of phosphorylated amino acids. However, the abundance of phosphorylated tyrosine in many cellular proteins increases tenfold following various activation processes. The importance of protein tyrosine phosphorylation has been established by demonstrating that it is an integral response in many different mitogenic receptor systems. Thus, many of the mitogenic receptor systems such as the EGF, PDGF and insulin receptors contain tyrosine kinase domains and become autophosphorylated on tyrosine upon binding of their ligands.[4] Others, such as the T-cell antigen receptor complex or the receptors for some hemopoietic growth factors may stimulate associated tyrosine kinases. Also, tyrosine-specific protein kinase activity has been described in many retroviral oncogenic proteins and cells transformed by these oncogenes contain elevated levels of phosphotyrosine.[4]

Many of the oncogenes found in mammalian oncogenic viruses encode tyrosine protein kinases that reside in the cellular cytoplasm. Others encode an enzyme that contains transmembrane domains and belong to a larger family of cell surface transmembrane receptors whose tyrosine phosphotransferase activity is stimulated by the binding of ligand to the extracellular domain and affects the regulation of cell growth.[5] The crucial role of tyrosine kinases in the signal transduction pathway of different regulatory circuits is evidenced by the observation that mutations that abolish their activity in many growth factor receptors and oncogenes also inactivate their mitogenic or transformative functions. Antibodies which are specific for phosphotyrosine enable a better approach in the analysis of the role of phosphorylation in signal transduction.

Reagent

Supplied as a lyophilized powder from a solution containing 1% BSA and 0.05% MIT in 0.01 M sodium phosphate buffered saline. After reconstitution, the conjugate concentration is 8-14 mg/mL. The molar ratio Ab/E = 0.6-1.5 and the enzyme activity is at least 250 U/mL.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the vial with 0.2 mL of distilled water.

Storage/Stability

Keep lyophilized reagent at 2-8 °C. For extended storage after reconstitution, freeze in working aliquots. For continuous use, the product may be stored at 2-8 °C for up to one month. Repeated freezing and thawing is not recommended.

Product Profile

ELISA

A minimum working dilution of 1:60,000 is determined using phosphotyrosine-BSA at 5 µg/mL.

Dot blot

A minimum working dilution of 1:40,000 is determined using phosphotyrosine-BSA at 40 ng/dot. (Substrate: AEC/H_2O_2).

Chemiluminescence Dot Blot

A minimum working dilution of 1:200,000 is determined using phosphotyrosine-BSA at 10 ng/dot. (Substrate: luminol + enhancer).

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

1. Alexander, D.R., and Cantrell, D.A., *Immunology Today*, **10**: 200 (1989).
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3. Wang, J.Y.J., *Anal. Biochem.*, **172**: 1 (1988).
4. Frackelton, A.R., et al., *Mol. Cell. Biol*. **3**: 1343 (1983).
5. Kamps, M.P., and Sefton, B.M., *Oncogene*, **2**: 305 (1988).

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