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Product Information

Monoclonal Anti-Phosphothreonine
Clone PTR-8
produced in mouse, ascites fluid

Catalog Number **P3555**

Product Description

Monoclonal Anti-Phosphothreonine (mouse IgG2b isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Phosphothreonine conjugated to KLH was used as the immunogen. The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-Phosphothreonine reacts against phosphorylated threonine both as free amino acid or when conjugated to carriers such as BSA or KLH using ELISA and dot blot. It does not react with non-phosphorylated threonine, phosphorylated tyrosine or serine, AMP or ATP. The antibody has been used for the localization of some phosphothreonine containing proteins using the immunoblotting method. Certain proteins known to contain phosphorylated threonine may not be recognized by this antibody due to steric hindrance of the recognition site.

Monoclonal Anti-Phosphothreonine may be used for the localization of phosphorylated threonine using various immunochemical assays such as ELISA, dot blot, and immunoblotting.

Protein phosphorylation and dephosphorylation are basic mechanisms for the modification of protein function in eukaryotic cells.[1] Phosphorylation is a rare post-translational event in normal tissue, however, the abundance of phosphorylated cellular proteins increases tenfold following various activation processes which are mediated through phosphotyrosine, phosphoserine or phosphothreonine (p-Tyr/p-Ser/p-Thr). Many different mitogenic systems, such as the EGF, PDGF and insulin receptor systems contain Tyr/Ser/Thr kinase domains which autophosphorylate specific Tyr/Ser/Thr residues upon binding of their ligands.[2] T cell antigen receptor complex or the receptors for some hemopoietic growth factors may stimulate associated kinases,[3] and cells transformed by viral oncogenes contain elevated levels of phosphorylated Tyr/Ser/Thr. An understanding of transformation by oncogenes and mitogenic processes of growth factors 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 phosphorylated proteins have been hampered by their low abundance and the problem of distinguishing the various types of phosphorylated proteins. The most common procedure is to label intact cells or small tissue fragments with ^{32}P and subsequently to isolate ^{32}P-labeled proteins by conventional biochemical methods. In order to identify the specific amino acids that undergo phosphorylation, additional long and tedious procedures for phosphoamino acid analysis are required. Immunoblotting of cellular proteins with antibodies directed against phosphoamino acids is advantageous as it does not involve ^{32}P labeling, and can therefore be employed to monitor alterations in phosphorylation of specific proteins as they occur in intact organs or even whole animals. Indeed, mono- and polyclonal antibodies directed against phosphorylated residues were generated and found useful as analytical and preparative tools[2,4] by enabling the identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

Reagent

Supplied as ascites fluid with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

For continuous use, store at 2-8 °C for a maximum of one month. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

Product Profile

Indirect ELISA: a minimum working dilution of 1:4,000 is determined using microtiter plates coated with phosphothreonine conjugated to BSA (10 μg/ml).

Indirect Immunoblotting: a minimum working dilution of 1:50 is determined using an extract of cultured human foreskin fibroblasts.

Note: In order to obtain optimum results, it is recommended that each individual user determine their optimum working dilutions by titration assay.

References

1. Hunter, T., and Cooper, J. A., *Annu. Rev. Biochem.*, **54**, 897 (1985).
2. Heffetz, D., et al., *Meth. Enzymol.*, **201**, 44 (1991).
3. Alexander, D. R., and Cantrell, D. A., *Immunol. Today*, **10**, 200 (1989).
4. Levine, L., et al., *J. Immunol Methods*, **124**, 239 (1989).

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