Product Information

Anti-Phosphothreonine Antibody, Mouse Monoclonal

Clone PTR-8, purified from hybridoma cell culture

P6623

Product Description

Anti-Phosphothreonine (mouse IgG2b isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells (NS1) and splenocytes from BALB/c mice immunized with phosphothreonine conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. No. ISO2.

Anti-Phosphothreonine reacts with phosphorylated threonine both as a 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 may be used for the detection of phosphorylated threonine using various immunochemical assays such as ELISA, dot blot,[1] immunoprecipitation,[2] immunocytochemistry[2] and immunoblotting.[3-5] Due to steric hindrance of the recognition site, this antibody may not recognize certain proteins known to contain phosphorylated threonine.

Protein phosphorylation and dephosphorylation are basic mechanisms for the modification of protein function in eukaryotic cells.[6] 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 that autophosphorylate specific Tyr/Ser/Thr residues upon binding of their ligands.[7] T cell antigen receptor complex or receptors for some hemopoietic growth factors may stimulate associated kinases,[8] 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. Immunoblotting of cellular proteins with antibodies directed against phosphoamino acids has been the method of choice for studying the role of certain phosphorylation events. Antibodies can 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[9], by enabling the identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

Reagents

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: ~2.5 mg/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.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze at –20 °C in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. 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

Immunoblotting: a working concentration of 5-10 µg/mL is determined using A431 cell extracts.

Indirect ELISA: a working concentration of 0.5-1.0 µg/mL is determined using Phosphothreonine BSA, Cat. No. P3842, 10 µg/mL, as the coating substrate.

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

References

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