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Product Information

Monoclonal Anti-Phosphothreonine-Biotin
clone PTR-8
produced in mouse, purified immunoglobulin

Catalog Number **B7661**

Product Description

Monoclonal Anti-Phosphothreonine (mouse IgG2b isotype) is derived from the PTR-8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice. Phosphothreonine conjugated to KLH was used as the immunogen. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The purified mouse immunoglobulin from ascites fluid is conjugated to biotin ε-amino caproic acid N-hydroxysuccinimide. This covalent coupling of biotin to the immunoglobulin allows for the binding of avidin, ExtrAvidin® or streptavidin bearing a variety of different labels.

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 several fold following various activation processes which are mediated through phospho-tyrosine, phosphoserine or phosphothreonine (p-Tyr/p-Ser/p-Thr). Many signal transduction pathways, such as the EGF, PDGF, and insulin receptor systems, contain Tyr/Ser/Thr kinase which phosphorylate specific Tyr/Ser/Thr residues upon binding of ligands to their receptors.[2] T cell antigen receptor complex or the receptors for some hemopoi-etic growth factors may stimulate these phosphorylation 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 their properties. 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 the whole animal. Indeed, mono- and polyclonal antibodies directed against phosphorylated residues have been generated and found useful as analytical and preparative tools[2,4] because they enable the rapid identification, quantification and, immuno-affinity isolation of phosphorylated cellular proteins.

Reagents

Supplied as a solution in phosphate buffered saline, with 1% BSA and 15 mM sodium azide as a preserva-tive.

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/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen 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.

Product Profile
Monoclonal Anti-Phosphothreonine reacts with 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-Biotin may be used as an analytical tool for the identification and quantification of threonine-phosphorylated proteins. Because avidin, streptavidin and ExtrAvidin® show high affinity interaction with biotin, the biotin-avidin system is an extremely effective detection tool in molecular biology, protein chemistry and immunology. With the high specificity of the biotinylated antibody to phosphothreonine, the stability of the biotin-avidin complex, and the availability of a variety of reagents (avidin, streptavidin or ExtrAvidin conjugated to FITC, TRITC, peroxidase or alkaline phosphatase), the detection and quantitation of threonine-phosphorylated proteins can be easily accomplished.

Direct ELISA: 1:40,000 using phosphothreonine-BSA at 10 µg/ml, and using ExtrAvidin-HRP at 2 µg/ml

Dot blot: 1:120,000

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

References

1. Hunter, T., and Cooper, *J., Annu. Rev. Biochem*., **54**, 897 (1985).
2. Heffetz, D., et al., *Methods 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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