Product No. F-3787
Lot 074H4826

Monoclonal Anti-Phosphothreonine FITC Conjugate

Purified Mouse Immunoglobulin
Clone PTR-8

Monoclonal Anti-Phosphothreonine (mouse IgG2b isotype) is derived from the PTR-8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A phosphothreonine-KLH conjugate was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). Protein A purified immunoglobulin from mouse ascites fluid is conjugated to FITC (isomer I) and then purified by gel filtration to remove free FITC. The conjugate is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.01% thimerosal as a preservative.

Specificity

FITC 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 immunoblotting. Certain proteins known to contain phosphorylated threonine may not be recognized by this antibody due to steric hindrance of the recognition site.

Description

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 phosphotyrosine, 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 hemopoietic 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 immunoaffinity isolation of phosphorylated cellular proteins.

Uses

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

F/P Molar Ratio: 4.3

Titer

1. DIBA (Fluorescent Dot Immunobinding Assay):
   1: 64 - 1:128

The titer of the conjugate was determined on a 2.5 - 5 µg dot of phosphothreonine-BSA bound to nitrocellulose.

2. PIFA (Particle Immunofluorescent Assay):
   1:32 - 1:64

The titer of the conjugate was determined using a 50 µl suspension of phosphothreonine-BSA-coated agarose using approximately 2 µg/ml of phosphothreonine-BSA.
In order to obtain best results it is recommended that each individual user determine their working dilution by titration assay.

Storage

For continuous use, store at 0-5°C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

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

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