

Product Information

MONOCLONAL ANTI-PHOSPHOSERINE

Clone PSR-45

Biotin Conjugate

Purified Mouse Immunoglobulin

Product Number **B 7911**

Product Description

Monoclonal Anti-Phosphoserine (mouse IgG1 isotype) is derived from the PSR-45 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice. Phosphoserine conjugated to KLH was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The purified mouse immunoglobulin from ascites fluid is conjugated to biotinamidohexanoic acid N-hydroxysuccinimide ester. 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 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 kinases 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 ³²P and subsequently to isolate ³²P-labeled proteins by conventional biochemical methods. In order to identify the specific amino acids that undergo phosphorylation, additional long and tedious procedures for phospho-amino acid analysis are required. Immunoblotting of cellular proteins with antibodies directed against phospho-amino acids is advantageous as it does not involve ³²P 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.

Reagents

The conjugate is provided as a liquid in 0.01 M phosphate buffered saline, pH 7.4, with 1% inactivated BSA and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for a maximum of one month. 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.

Product Profile

Monoclonal Anti-Phosphoserine reacts with phosphorylated serine 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 serine, phosphorylated tyrosine or threonine, AMP, or ATP. The antibody has been used for the localization of some phosphoserine containing proteins with the immunoblotting method. Certain proteins known to contain phosphorylated serine may not be recognized by this antibody due to steric hindrance of the recognition site.

Biotin Conjugated Monoclonal Anti-Phosphoserine may be used as an analytical tool for the identification and quantification of serine-phosphorylated proteins. Because avidin, streptavidin, and ExtrAvidin show high affinity 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 phosphoserine, 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 serine-phosphorylated proteins can be easily accomplished.

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., *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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