

## Product Information

### **MONOCLONAL ANTI-PHOSPHOSERINE CLONE PSR-45 Mouse Ascites Fluid**

Product Number **P 3430**

#### **Product Description**

Monoclonal Anti-Phosphoserine (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. 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 assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

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

Protein phosphorylation and dephosphorylation are basic mechanisms for the modification of protein function in eukaryotic cells.<sup>1</sup> 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.<sup>2</sup> T cell antigen receptor complex or the receptors for some hemopoietic growth factors may stimulate associated kinases,<sup>3</sup> 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 <sup>32</sup>P and subsequently to isolate <sup>32</sup>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 <sup>32</sup>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<sup>2,4</sup> by enabling the identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

#### **Reagents**

The product is provided as ascites fluid with 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 up to 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

1. A minimum working dilution of 1:4,000 is determined by indirect ELISA using microtiter plates coated with phosphoserine conjugated to BSA (10 µg/ml).
2. A minimum working dilution of 1:500 is determined by indirect immunoblotting using an extract of rat brain cortex.

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., Methods Enzymol., **201**, 44 (1991).
3. Alexander, D., and Cantrell, D., Immunol. Today, **10**, 200 (1989).
4. Levine, L., et al., J. Immunol. Methods, **124**, 239 (1989).

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