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## Product Information

### Monoclonal Anti-Phosphoserine

#### Clone PSR-45

Purified Mouse Immunoglobulin

Product Number **P 5747**

#### 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 a BALB/c mouse immunized with phosphoserine conjugated to KLH. The isotype is determined using Sigma ImmunoType Kit (Product No. ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product No. ISO-2).

Monoclonal Anti-Phosphoserine recognizes 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 detection of some phosphoserine containing proteins using immunoblotting<sup>1,2,8-10</sup> and dot blotting.<sup>3</sup> Due to steric hindrance this antibody may not recognize certain proteins known to contain phosphorylated serine.

Protein phosphorylation and dephosphorylation are basic signaling mechanisms that modify protein function in eukaryotic cells.<sup>4</sup> Phosphorylation is a rare post-translational event in normal tissues, however, the abundance of phosphorylated cellular proteins increases several fold following various activation processes. The main amino acids that are phosphorylated are tyrosine, serine, or threonine (p-Tyr/p-Ser/p-Thr) each having specific kinases that phosphorylate them and specific phosphatases that dephosphorylate them. Different growth factor receptors, such as the EGF-R, PDGF-R, and insulin receptor, contain Tyr/Ser/Thr residues that are autophosphorylated upon binding to their ligands.<sup>5</sup> Ligand binding of other receptors, like the T cell antigen receptor complex or receptors for some hematopoietic growth factors, stimulate downstream associated kinases.<sup>6</sup> 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. Immunoblotting of cellular proteins with antibodies directed against phosphoamino acids is advantageous over <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 in whole animals. Indeed, mono- and polyclonal antibodies directed against phosphorylated residues were generated and found useful as analytical and preparative tools<sup>2,7</sup> by enabling the identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

#### Reagent

The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as preservative.

Antibody Concentration: approx. 1.0 mg/mL

#### 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 hazardous and safe handling practices.

#### Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage freeze 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. Working dilution samples should be discarded if not used within 12 hours.

### Product Profile

A working concentration of 2.5-5 µg/ml is determined by immunoblotting, using total rat brain extract.

A working concentration of 0.3-0.6 µg/ml is determined by indirect ELISA using microtiter plates coated with phosphoserine conjugated to BSA (Product No. P 3717, 10 µg/ml).

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

### References

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