

Product Information

MONOCLONAL ANTI-PHOSPHO-PAK1 (pThr²¹²) CLONE PK-18

Purified Mouse Immunoglobulin

Product Number **P 3237**

Product Description

Monoclonal Anti-Phospho-PAK1 (pThr²¹²) (mouse IgG1 isotype) is derived from the PK-18 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 203-217 (pThr²¹²) of human PAK1,¹ conjugated to keyhole limpet hemocyanin (KLH). 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).

Monoclonal Anti-Phospho-PAK1 (pThr²¹²) reacts specifically with human PAK1 phosphorylated at Thr²¹², and does not detect the unphosphorylated epitope.¹ The product is useful in ELISA, immunoblotting¹ (68 kDa, for the endogenous molecule, and a variety of molecular mass sizes in transfection products), and immunocytochemistry (4% paraformaldehyde, 37 °C).¹ Reactivity has been observed with human, rat, and mouse PAK1.¹

Phosphorylation is a ubiquitous cellular regulatory mechanism utilized by all tissues and species. A large number of cellular processes are regulated by a reversible conformational covalent modification of proteins that results from the reversible phosphorylation of specific serine, threonine or tyrosine residues.

PAK1 (p21-activated kinase 1) belongs to the serine/threonine protein kinases family, with high homology to the *Sacharomyces cerevisiae* kinase Ste20. PAK1 regulates cell morphology and polarity in most, if not all, eukaryotic cells.² PAK1 (68 kDa), has a C-terminus serine/threonine kinase domain, an N-terminus CRIB (Cdc42/Rac interactive binding) domain [also called PBD (p21-binding domain), and GBD (GTPase binding domain)]. It is activated by binding to the Rho family small G proteins Rac and Cdc42. Indeed, in cells stimulated by growth factors, apoptosis or otherwise, PAK1 is found in a complex with Rac/Cdc42 and GTP. This association causes the autophosphorylation of Thr⁴²³ in the activation loop of

PAK1.³ Once phosphorylated, PAK1 dissociates from Rac/Cdc42 complex and activates downstream targets like JNK (c-Jun N-terminal protein kinase) and p38 MAP kinase. These kinases regulate several cellular processes, among them proliferation, differentiation, cellular morphology and oncogenesis.

In neurons, membrane-associated active PAK1 is regulated by the p35/Cdk5 kinase, both by association and phosphorylation of Thr²¹², which is essential for the proper regulation of the cytoskeleton during neurite outgrowth and remodelling.^{1, 4} Two other closely related members of the PAK family of kinases, PAK2 and PAK3, do not have a p53/Cdk5 phosphorylation site at the position equivalent to Thr²¹². Therefore, in neurons, phosphorylation of PAK1 on Thr²¹² may regulate a specific signaling pathway, distinguishing it from other members of this family of kinases.^{1, 5} Indeed, in neuronal growth cones, PAK1 phosphorylating on Thr²¹² localizes to actin and tubulin rich areas, suggesting a role in regulating growth cone dynamics.¹ The crystal structure of PAK1 domains reveals that Thr²¹² resides in a flexible region, conserved in human, rat and mouse.¹

PAK1 also regulates cytoskeletal dynamics by decreasing MLCK (myosin light chain kinase) activity and MLC phosphorylation.^{2, 6} Finally, PAK1 is reported to be a crucial signaling molecule, involved in the transcription factor NFκB activation by multiple stimuli.⁷

Monoclonal antibodies reacting specifically with phospho-PAK1 (pThr²¹²) are useful tools in the study of the roles of PAK1 phosphorylation in many pathways, especially in neuronal differentiation.

Reagent

Monoclonal anti-Phospho-PAK1 (pThr²¹²) is supplied as a 0.2 ml solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: approximately 2 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

Store at 2-8 °C for up to one month.

For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is also 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 0.2-0.4 µg/ml is determined by immunoblotting, using a whole extract of transfected COS-7 (monkey kidney) cells, expressing human PAK1 phosphorylated at Thr²¹².

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

References

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