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ProductInformation

Anti-phospho-PAK1/2/3 [pThr^{423}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 7746**

Product Description

Anti-phospho-PAK1/2/3 [pThr^{423}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of PAK that contains threonine 423 as immunogen. The sequence is conserved throughout many species for all three isoforms of PAK. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide.

The antibody detects human PAK1, PAK2, and PAK3. Other species (100% homologous) have not been tested, but are expected to react. It has been used in immunoblotting applications.

p21-activated kinase (PAK) is actually a family of serine/threonine protein kinases, members of which are activated by small molecular weight GTPases. The three most common isoforms are PAK1, PAK2, and PAK3 (also known as α-PAK, γ-PAK, and β-PAK, respectively). These kinases contain numerous regulatory elements that trigger diverse signaling processes such as those initiated by activated GTPases, interaction with Src homology 3 (SH3) domains, and caspase-mediated proteolytic cleavage.

Autophosphorylation of threonine 423 (threonine 402 for PAK2 and threonine 421 for PAK3), catalyzed by Cdc42, is required for activation of PAK.

Reagent

Anti-phospho-PAK1/2/3 [pTh^{423}] is provided as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

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

Store at –20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

Product Profile

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using His-tagged wild-type and S139A/T421A double mutant PAK3.

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

Results

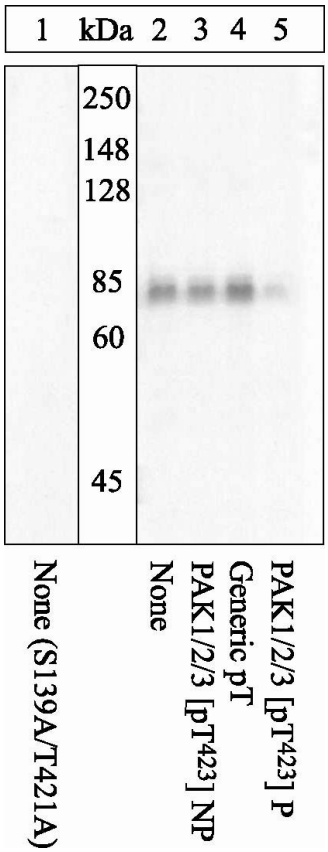
Peptide Competition

1. Extracts prepared from his-tagged S139A/T421A double mutant (Lane 1) and wild-type PAK3 (Lanes 2-5) were resolved by SDS-PAGE on a 10% Tris-glycine gel, and transferred to PVDF.
2. Membranes, were incubated with a 5% BSA-TBST buffer overnight at 4 °C, in order to block non-specific sites.
3. Subsequently the membranes were incubated as follows:

Lane 1, 2	no peptide
Lane 3	the non-phosphopeptide corresponding to the immunogen
Lane 4	a generic peptide containing phosphorylated tyrosine
Lane 5	Immunogen

4. All lanes were incubated with 0.50 µg/mL PAK1/2/3 [pTh^{423}] antibody
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data show that only the peptide corresponding to PAK1/2/3 [pTyr^{423}] blocks the antibody signal, and phosphorylation with the wild-type but not the mutant protein, thereby demonstrating the specificity of the antibody.



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