

Rabbit (polyclonal) Anti-PAK1/2/3 [pT⁴²³] Phosphospecific Antibody, Unconjugated

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Rev. 1.00

Catalog Number: 44942G

Store at 2 to 8°C (short-term), or -20°C (long-term)

Clonality: Polyclonal
Quantity: 10 mini-blot size
Volume: 100 µL

Host/Class: Rabbit
Reactivity: Human PAK1, PAK2, and PAK3

Product Description

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. During development reactivity was confirmed with human PAK1, PAK2 and PAK3. Other species (100% homologous) have not been tested.

Product Specifications

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human PAK that contains threonine 423 (for PAK1). The sequence is conserved across many species for all three main isoforms of PAK.

Lot: See product label

Product Applications

Application	Species	Test Material	Concentration
Western Blotting	Human	Cdc42-activated, His-tagged wild-type and S139A/T421A double mutant recombinant PAK3 proteins	1:1000

Storage and Handling

Store at -20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at -20°C. For short term storage (up to one week), 2 to 8°C is sufficient.

Stability

When stored as instructed, expires one year from date of receipt unless otherwise indicated on product label.

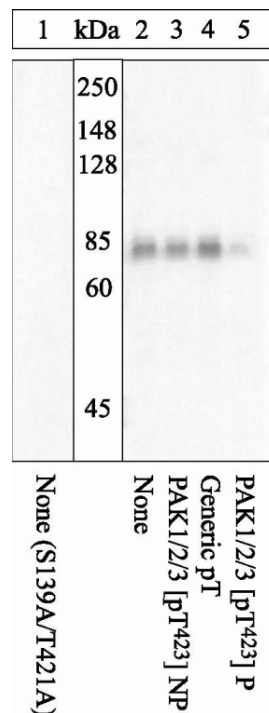


Figure 1 Peptide Competition

Cdc42-activated, His-tagged recombinant S139A/T421A double mutant (lane 1) and wild-type PAK3 (lanes 2–5) were added to background extracts and resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer overnight at 4°C, then incubated with the PAK1/2/3 [pT⁴²³] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (lanes 1, 2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (lane 3), a generic phosphothreonine-containing peptide (lane 4), or the phosphopeptide immunogen (lane 5). After washing, the membrane was incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and bands were detected using the Western-Star™ method.

The data show that only the phosphopeptide corresponding to PAK1/2/3 [pT⁴²³] blocks the antibody signal, and detection of the wild-type but not the mutant protein is detected by the antibody, demonstrating the specificity of the antibody.

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Storage Buffer

Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (± 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier. Contains 0.05% sodium azide.

Caution: Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Properly dispose of solutions containing sodium azide.








Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

Certificate of Analysis

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Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		
	Consult instructions for use		Caution, consult accompanying documents		

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