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Antibodies to Protein Kinases.

Antibody to p21-activated protein kinase (p65^{PAK}). Cat # PAK-101AP

A brain serine threonine protein kinase is a target for p21 ras related proteins Cdc42 and Rac1. p21-activated kinases (PAKs) bind to and are activated by Rho family GTPases such as Cdc42 and Rac. These GTPases play key roles in regulating cell polarity, stress responses, and cell cycle progression (1). Stoichiometric analyses using purified Rac and PAK suggest that Rac binds to PAK with high affinity in a 1:1 ratio. The kinase complexes specifically with aGTP bound activated p21, inhibiting the p21 activity and leading to activation and auto-phosphorylation of p65pak (2). Three isoforms of p21-activated serine/threonine kinases, PAK68, PAK65 and PAK62, were found in both cytosolic & membrane fraction, & their contents were significantly increased during induced retinoic acid differentiation of human promyelocytic leukemia HL60 cell (3). The content of PAK65 or Rac is not evident in plasma membrane fraction of cells from undifferentiated cells (2).

Constitutively activated Pak1 also suppress the activity of scavenger receptor class B, type 1 (SB-B1) in macrophage cell line RAW264.7, an action similar to LPS (4). Mutational studies with PAK1 also suggest that SR-B1 receptor down regulation involves binding to Rac and Cdc42. In mammalian cells, hyper osmotic shocks can induce apoptotic cell death. It is suggested that hyper osmotic shock can induce cleavage for PAK2 to produce a 36kDa kinase, which is the result of DNA fragmentation and activation of ICE/CED-3 family cysteine proteases termed caspases-3.

The p21 activated PAK-selective antibodies were generated against synthetic peptide corresponding to residues 484-502 corresponding to human lymphocyte p65PAK. The antibodies to p65PAK (PAK-101AP) are affinity purified over immobilized antigen based chromatography. The purified antibodies are stabilized in antibody stabilization buffer containing preservatives. FabGennix Int. Inc., will also provide Western blot positive controls for human p65PAK in ready-to-use buffer. Please enquire about pricing and availability of the Western blot positive controls. Other kinase related antibodies that are carried by FabGennix Int. Inc. include p²¹ activated protein kinase (p65^{pak}), Anti-calmodulin dependent kinase gamma, Anti-MER tyrosine kinase (MERTK) and Anti-auto activated protein kinase. *FabGennix Inc.* will also conjugate antibodies with fluorescent probes, magnetic beads or to various affinity matrices upon request at a reasonable cost.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	price
PAK-101AP	Rabbit	Affinity purified antibodies	R, M, H, *C, *monk	50 ug	205
PC-PAK	Human	Western blot controls	n/a	5 appl.	inquire

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; * not all variants are labeled equally

Immunogen: Synthetic peptide from lymphocyte p21 PAK65kinase corresponding to position 484-502. This peptide sequence is conserved in many other animal species including rat, mouse, monkey etc.

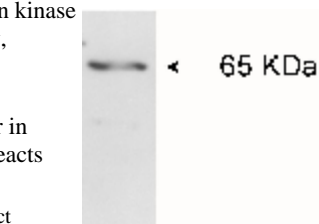
Concentration: PAK-101AP Ig concentration 1-1.25 mg/ml in 50% antibody stabilization buffer.

Applications: Antibody PAK-101AP is ideal for IMM/WB applications for detection of ?auto activated protein kinase in various tissues of several animal species. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay in his/her Laboratory. WB > 1:250; IMM & i.p pull-down assays: > 1:100

Reactivity: This antibody detects a single band of approximately 65 kDa of PAK in Hek 293 cell extracts or in Western blot positive control for PAK (Cat # PC-PAK) samples. The antibody does not cross reacts with other proteins from the protein kinase family or with other proteins.

Protocols: Standard protocol for various applications (WB; IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

Form/Storage: The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide. The affinity-purified antibodies are purified on antigen-sphere affinity column and supplied in antibody stabilization buffer containing preservatives. For long-term storage of antibodies, store at -20°C. FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µ filter after every use for long-term storage.



Western blot using Western blot control (PC-PAK) and 1:250 dil PAK-101AP

References:

1. Sells MA., Barret JT., Caviston J., Ottie S., Leberer E. chernoff J. J. Biol. Chem. 1998; 273, 18490-18498.
2. Manser E., Leung T., Salihuddin H., Zhao Z. S., Lim L. Nature 1994, 367(6458); 40-46.
3. Nisimoto Y., and Ogawa H. 2002, Europ. J. Biochem. 269, 2622-, 2628.
4. Hullinger TG., Panek RL., Karathanasis SK. J. Biol. Chem. 2001; 276, 46807-46814.

For users who may require large amounts of PAK-101AP, please enquire about bulk material discounts.

This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

092602-0020SF100 ge02-rev10.00

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