

#2601 Store at -20°C

Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody

100 µl
 (10 western blots)



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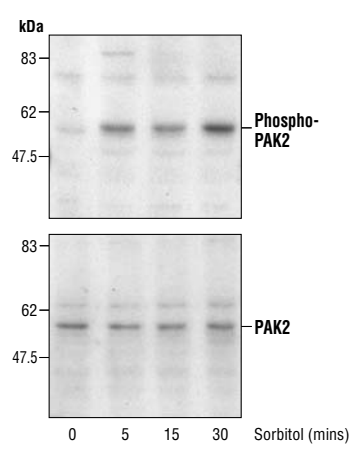
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, Guinea Pig, (R)	61 to 67 (PAK2), 68 to 74 (PAK1/3)	Rabbit**

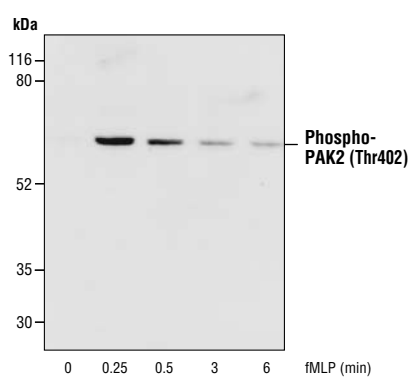
Background: The p21-activated kinase (PAK) family of serine/threonine kinases is engaged in multiple cellular processes, including cytoskeletal reorganization, MAPK signaling, apoptotic signaling, control of phagocyte NADPH oxidase and growth factor-induced neurite outgrowth (1,2). Several mechanisms that induce PAK activity have been reported. Binding of Rac/cdc42 to the CRIB (or PBD) domain near the amino terminus of PAK causes autophosphorylation and conformational changes in PAK (1). Phosphorylation of PAK1 at Thr423 by PDK induces activation of PAK1 (3). Several autophosphorylation sites have been identified, including serines 199 and 204 of PAK1 and serines 192 and 197 of PAK2 (4,5). Because the autophosphorylation sites are located in the amino-terminal inhibitory domain, it has been hypothesized that modification in this region prevents the kinase from reverting to an inactive conformation (6). Research indicates that phosphorylation of Ser144 of PAK1 or Ser139 of PAK3 (located in the kinase inhibitory domain) affects kinase activity (7). Phosphorylation of Ser21 of PAK1 or Ser20 of PAK2 regulates binding with the adaptor protein Nck (8). More recently identified family members including PAK4, PAK5 and PAK6 have lower sequence similarity with PAK1-3 in the amino-terminal regulatory region (9). Phosphorylation of Ser474 of PAK4, a site analogous to Thr423 of PAK1, may play a pivotal role in regulating the activity and function of PAK4 (10).

Specificity/Sensitivity: Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody detects endogenous PAK1, PAK2 and PAK3 only when phosphorylated at Thr423, Thr402 and Thr421, respectively. The antibody does not cross-react with phosphorylated PAK4, PAK5 or PAK6. The antibody does cross-react with phospho-Mst1 (Thr183) or phospho-Mst2 (Thr180).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr423 of human PAK1. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HeLa cells treated with 0.4 M sorbitol for indicated times, using Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody (upper) or PAK2 antibody (lower).



Western blot analysis of extracts from guinea pig neutrophils stimulated with 1 µM fMLP for indicated times, using Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody (Provided by Drs. Qian Zhan and John Badwey, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Massachusetts.)

Entrez-Gene ID #5058, 5062, 5063
Swiss-Prot Acc. #Q13153, Q13177, O75914

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

***Species cross-reactivity is determined by Western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:
 Western Blotting 1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Selected Application References:

Zhan, Q. et al. (2003) p21-Activated Kinase 2 in Neutrophils Can Be Regulated by Phosphorylation at Multiple Sites and by a Variety of Protein Phosphatases. *The Journal of Immunology* 171, 3785–3793. Application: W.

Rudrabhatla, R.S. et al. (2003) Modulation of Myosin Light-Chain Phosphorylation by p21-Activated Kinase 1 in Escherichia coli Invasion of Human Brain Microvascular Endothelial Cells. *Infection and Immunity* 71 (5), 2787–2797. Application: W.

Weber, D.S. et al. (2004) Phosphoinositide-Dependent Kinase 1 and p21-Activated Protein Kinase Mediate Reactive Oxygen Species-Dependent Regulation of Platelet-Derived Growth Factor-Induced Smooth Muscle Cell Migration. *Circulation Research* 94, 1219–1226. Application: W.

Harfouche, R. et al. (2005) Roles of reactive oxygen species in angiopoietin-1/tie-2 receptor signaling. *FASEB J* 19, 1728–30. Application: W.

Background References:

- (1) Knaus, U.G. and Bokoch, G.M. (1998) *Int. J. Biochem. Cell Biol.* 30, 857–862.
- (2) Daniels, R.H. et al. (1998) *EMBO J.* 17, 754–764.
- (3) King, C.C. et al. (2000) *J. Biol. Chem.* 275, 41201–41209.
- (4) Manser, E. et al. (1997) *Mol. Cell. Biol.* 17, 1129–1143.
- (5) Gatti, A. et al. (1999) *J. Biol. Chem.* 274, 8022–8028.
- (6) Lei, M. et al. (2000) *Cell* 102, 387–397.
- (7) Chong, C. et al. (2001) *J. Biol. Chem.* 276, 17347–17353.
- (8) Zhao, Z. et al. (2000) *Mol. Cell. Biol.* 20, 3906–3917.
- (9) Abo, A. et al. (1998) *EMBO J.* 17, 6527–6540.
- (10) Qu, J. et al. (2001) *Mol. Cell. Biol.* 21, 3523–3533.

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5%w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.