

#3241 Store at -20°C

# Phospho-PAK4 (Ser474)/PAK5 (Ser602)/PAK6 (Ser560) Antibody

✓ 100 µl (10 Western mini-blot)

Orders ■ 877-616-CELL (2355) orders@cellsignal.com  
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

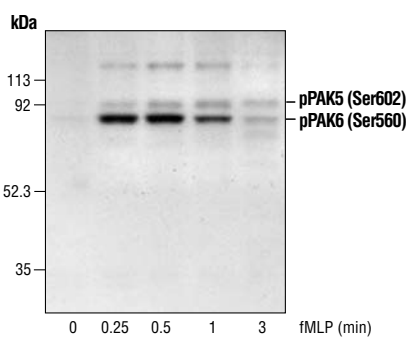
Applications	Species Cross-Reactivity*		Molecular Wt.	Source
	W	H, Guinea Pig		
			72 kDa (PAK4), 82 kDa (PAK6), 90 kDa (PAK5)	Rabbit

**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-PAK4 (Ser474)/PAK5 (Ser602)/PAK6 (Ser560) Antibody detects endogenous levels of PAK4, PAK5 and PAK6 only when phosphorylated at serine 474, 602, or 560, respectively. The antibody does not cross-react with phosphorylated PAK1, PAK2 or PAK3.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser474 of human PAK4. Antibodies are purified by protein A and peptide affinity chromatography.

**Selected Application References:**  
Schrantz, N. et al. (2004) Mechanism of p21-activated Kinase 6-mediated Inhibition of Androgen Receptor Signaling. *J. Biol. Chem.* 279, 1922–1931. Application: W.  
Kaur, R. et al. (2005) Activation of p21-activated kinase 6



Western blot analysis of extracts from guinea pig neutrophils stimulated with fMLP for the indicated times using Phospho-PAK4 (Ser474)/PAK5 (Ser602)/PAK6 (Ser560) Antibody. (Provided by Drs. Qian Zhan and John Badwey, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Massachusetts.)

by MAP kinase kinase 6 and p38 MAP kinase. *J Biol Chem* 280, 3323–30. Application: W.

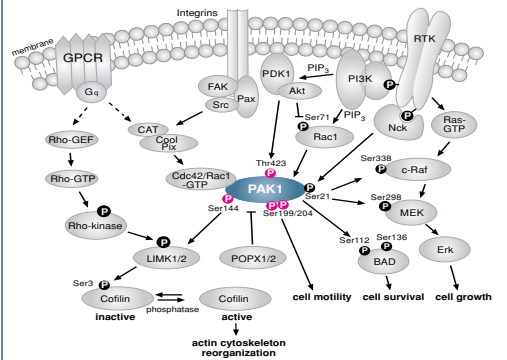
- Background References:**
- (1) Knaus, U.G. and Bokoch, G.M. (1998) *Int. J. Biochem. Cell Biol.* 30, 857–862.
  - (2) Daniels, R.H. et al. (1998) *EMBO J.* 17, 754–764.
  - (3) King, C.C. et al. (2000) *J. Biol. Chem.* 275, 41201–41209.
  - (4) Manser, E. et al. (1997) *Mol. Cell. Biol.* 17, 1129–1143.
  - (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.

**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.

**Recommended Antibody Dilutions:**  
Western Blotting 1:1000

- Companion Products:**  
Phospho-Rac1/cdc42 (Ser71) Antibody #2461  
PAK4 Antibody #3242  
Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody #2601  
PAK1 Antibody #2602  
PAK1/2/3 Antibody #2604  
Phospho-PAK1 (Ser199/204)/PAK2 (Ser192/197) Antibody #2605  
Phospho-PAK1 (Ser144)/PAK2 (Ser141) Antibody #2606  
Phospho-PAK2 (Ser20) Antibody #2607  
PAK2 Antibody #2608  
PAK3 Antibody #2609  
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071  
Anti-rabbit IgG, HRP-linked Antibody #7074  
Prestained Protein Marker, Broad Range (Premixed Format) #7720  
Biotinylated Protein Ladder #7727  
20X LumiGLO® Reagent and 20X Peroxide #7003



**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 IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM  
Z—zebra fish B—bovine All—all species expected

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.