

#4750 Store at -20°C

PAK 1/2/3 Antibody Sampler Kit



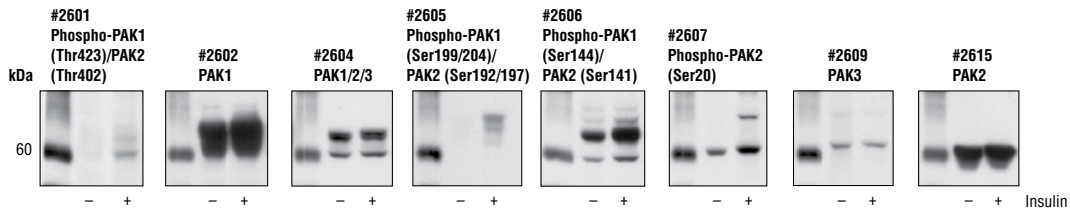
1 Kit
 (8 x 40 μl)

Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
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New 05/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Applications	Species Cross-Reactivity	Mol. Wt.	Source
Phospho-PAK1 (Ser144)/PAK2 (Ser141) Antibody	2606	40 μl	W	H, (M, R)	61-67 kDa (PAK2), 68-74 kDa (PAK1/3)	Rabbit
Phospho-PAK1 (Ser199/204)/PAK2 (Ser192/197) Antibody	2605	40 μl	W	H, M, (R)	61-67 kDa (PAK2), 68-74 kDa (PAK1/3)	Rabbit
Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody	2601	40 μl	W	H, M, (R)	61-67 kDa (PAK2), 68-74 kDa (PAK1/3)	Rabbit
Phospho-PAK2 (Ser20) Antibody	2607	40 μl	W	H, (M, R)	61-67 kDa	Rabbit
PAK1 Antibody	2602	40 μl	W, IP, IHC-P	H, M, R, Mk	68 kDa	Rabbit
PAK2 (C17A10) Rabbit mAb	2615	40 μl	W	H, M, Mk	61 kDa	Rabbit
PAK3 Antibody	2609	40 μl	W, IP	H, M, (R)	65 kDa	Rabbit
PAK1/2/3 Antibody	2604	40 μl	W	H, M, R, Mk	61 kDa (PAK2), 68 kDa (PAK1/3)	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 μl				Goat



Western blot analysis of extracts from C2C12 cells, untreated or insulin-treated (100 nM) for 20 minutes, using: Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody #2601; PAK1 Antibody #2602; PAK 1/2/3 Antibody #2604; Phospho-PAK1 (Ser199/204)/PAK2 (Ser192/197) Antibody #2605; Phospho-PAK1 (Ser144)/PAK2 (Ser141) Antibody #2606; Phospho-PAK2 (Ser20) Antibody #2607; PAK3 Antibody #2609; and PAK2 Rabbit mAb #2615.

Description: The PAK Antibody Sampler Kit provides an economical means to evaluate the activation status of PAK1, 2, and 3. This kit includes enough primary and secondary antibodies to perform four western blots with each antibody.

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).

Specificity/Sensitivity: Phospho-PAK2 (Ser20) Antibody, PAK1 Antibody, PAK2 (C17A10) Rabbit mAb, PAK3 Antibody and PAK1/2/3 Antibody detect endogenous levels of their target protein(s) and do not cross-react with other PAK family members. Phospho-PAK1 (Ser199/204)/PAK2 (Ser192/197), Phospho-PAK1 (Ser144)/PAK2 (Ser142), and Phospho-PAK1 (Thr423)/PAK2 (Thr402) antibodies, which may also detect Ser200/205, Ser139, and Thr421 of phosphorylated PAK3, respectively. Phospho-PAK1 (Thr423)/PAK2 (Thr402) Antibody cross-reacts with phospho-Mst1 (Thr183) or phospho-Mst2 (Thr180).

Source/Purification: Phospho-specific antibodies are produced by immunizing animals with synthetic phosphopeptides (KLH-coupled) corresponding to residues surrounding Ser144, Ser199/204 and Thr423 of human PAK1, and Ser20 of human PAK2. PAK1 Antibody and PAK2 Antibody are produced by immunizing animals with synthetic peptides (KLH-coupled) corresponding to residues surrounding the aminotermini of PAK1 and PAK2. PAK3 Antibody and PAK1/2/3 Antibody is produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to residues at the carboxy-terminus of human PAK3 and PAK1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu\text{g}/\text{ml}$ BSA and 50% glycerol. Store at -20°C . Do not aliquot the antibodies.

Recommended Antibody Dilutions:
 Western blotting 1:1000
 See www.cellsignal.com for individual component dilutions and additional application protocols.

- Companion Products:**
 Phospho-PAK4 (Ser474)/PAK5 (Ser602)/PAK6 (Ser560) Antibody #3241
 PAK4 Antibody #3242
 PAK2 Antibody #2608
 Nonfat Dry Milk #9999
 BSA #9998
 Anti-rabbit IgG, HRP-linked Antibody #7074
 20X LumiGLO[®] Reagent and 20X Peroxide #7003
 Phototope[®]-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 Prestained Protein Marker, Broad Range (Premixed Format) #7720
 Biotinylated Protein Ladder Detection Pack #7727

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine Dg—Dog All—all species expected
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

**Background References:**

- (1) Knaus, U.G. and Bokoch, G.M. (1998) *Int J Biochem Cell Biol* 30, 857-62.
- (2) Daniels, R.H. et al. (1998) *EMBO J* 17, 754-64.
- (3) King, C.C. et al. (2000) *J Biol Chem* 275, 41201-9.
- (4) Manser, E. et al. (1997) *Mol Cell Biol* 17, 1129-43.
- (5) Gatti, A. et al. (1999) *J Biol Chem* 274, 8022-8.
- (6) Lei, M. et al. (2000) *Cell* 102, 387-97.
- (7) Chong, C. et al. (2001) *J Biol Chem* 276, 17347-53.
- (8) Zhao, Z.S. et al. (2000) *Mol Cell Biol* 20, 3906-17.

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[®]-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[®] 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 cm²) 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[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] 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[®] incubation and declines over the following 2 hours.