Store at -80°C

#7636

HTScan® PAK2 Kinase Assay Kit

☑100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Products Included	Products #	Kit Quantity
Phospho-Tyrosine Hydroxylase (Ser40) Antibody	2791	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
Tyrosine Hydroxylase (Ser40) Biotinylated Peptide	1132	1.25 ml
PAK2 Kinase (recombinant, human)	7635	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human PAK2 kinase. It includes active PAK2 kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine/threonine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: RQS*LI

Molecular Weights: Peptide substrate, Biotin-peptide: 2,326 Daltons. GST-PAK2 Kinase: 88 kDa.

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).

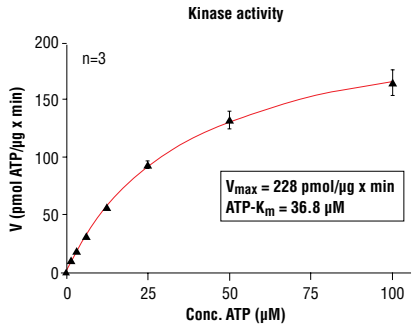

Figure 1. PAK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20.000, Substrate: Tetra (LRRWSLG), 5 µg/50 µl, recombinant PAK1: 50 ng/50 µl.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human PAK2 (Met1-Arg534) (GenBank Accession No. NM_002577) with an amino-terminal GST tag. The protein was purified by one-step affinity purification using glutathione-agarose.

Quality Control: The substrate peptide was selected using Serine/threonine Kinase Substrate Screening Kit #7400 to screen for PAK2 kinase substrates. Phospho-Tyrosine Hydroxylase (Ser40) Antibody (#2791) was used for detection. The quality of the biotinylated peptides was evaluated by reverse-phase HPLC and mass spectrometry.

Purified PAK2 kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the PAK2 kinase was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose-dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify PAK2 activity using the PAK2 substrate peptide provided in this kit. PAK2 sensitivity to the inhibitor staurosporine was measured using the PAK2 substrate peptide provided in this kit [Fig.5].

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Serine/Threonine Kinase Substrate Screening Kit #7400

PAK2 Kinase #7635

Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791

Tyrosine Hydroxylase (Ser40) Biotinylated Peptide #1132

Staurosporine #9953

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.

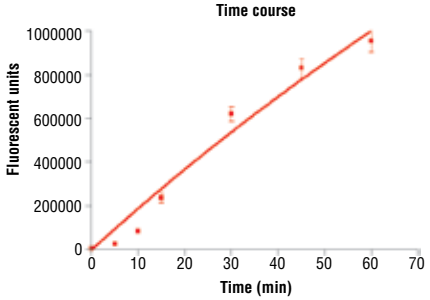

Figure 2. Time course of PAK2 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of PAK2 substrate peptide (#1132) by PAK2 kinase. In a 50 µl reaction, 50 ng PAK2 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

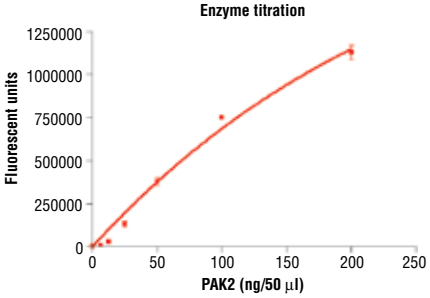

Figure 3. Dose dependence curve of PAK2 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of substrate peptide (#1132) by PAK2 kinase. In a 50 µl reaction, increasing amounts of PAK2 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

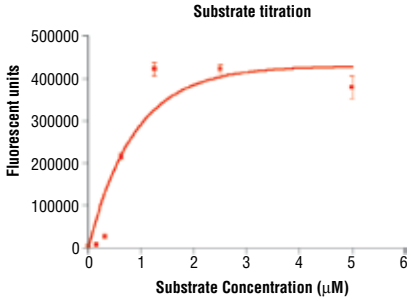

Figure 4. Peptide concentration dependence of PAK2 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of substrate peptide (#1132) by PAK2 kinase. In a 50 µl reaction, 50 ng of PAK2 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

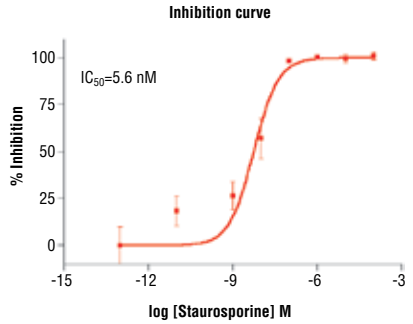

Figure 5. Staurosporine inhibition of PAK2 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of PAK2 substrate peptide (#1132) by PAK2 kinase. In a 50 µl reaction, 50 ng PAK1, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® PAK2 Kinase Assay Kit

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [1 ml 10X Kinase Buffer 250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Transfer 1.2 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 4 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng PAK2 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody, Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

***NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com