

PAK3 Kinase

✓ 5 µg

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 02/07

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

Description: Purified recombinant full length human PAK3 (Met1-Arg544) kinase, supplied as a GST fusion protein.

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).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system using sf9 cells and a recombinant virus encoding for the full length human PAK3 (Met1- Arg544) (GenBank Accession No. NM_002578) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-PAK3 fusion protein is 90 kDa. The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. PAK3 kinase activity was determined using a radiometric assay [Fig.2].

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: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

- Kinase Buffer (10X) #9802
- ATP (10 mM) #9804
- Serine/Threonine Kinase Substrate Screening Kit #7400

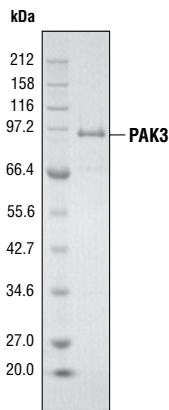


Figure 1. The purity of the GST-PAK3 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

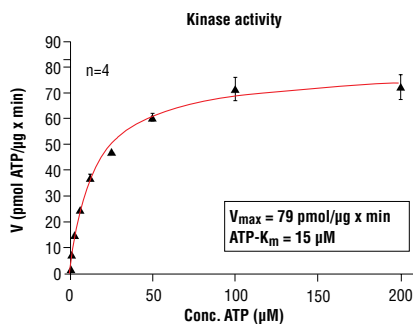


Figure 2. PAK3 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 3 µM Na-orthovanadate, 1.2 mM DTT, 50 µg/µl PEG20,000, ATP variable, Substrate: Tetra (LRRWSLG) 2 µg/µL, and recombinant PAK3: 200 ng/50 µl.

Protocol for PAK3 Kinase Assay

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. Kinase Buffer (5X)**
300 mM HEPES-NaOH, pH 7.5
15 mM $MgCl_2$
15 mM $MnCl_2$
15 μ M Na-orthovanadate
6 mM DTT
250 μ g/ μ l PEG_{20,000}
- 2. ATP (10 mM) #9804**
- 3. ^{32}P - γ ATP**
- 4. Tetra (LRRWSLG) (5 μ g/ μ l)**

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute PAK3 protein to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted PAK3 kinase solution, 10 μ l Tetra (5 μ g/ μ l), and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 60 mM HEPES-NaOH, pH 7.5
 - 3 mM $MgCl_2$
 - 3 mM $MnCl_2$
 - 3 μ M Na-orthovanadate
 - 1.2 mM DTT
 - 40 ng/ μ l PEG_{20,000}
 - 20 μ g/ μ l Tetra
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.