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Catalog #	Aliquot Size
P02-10G-05	5 µg
P02-10G-10	10 µg

PAK1/CDC42, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog # P02-10G
Lot # A1460-5

Product Description

Recombinant full-length human PAK1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. Combined with CDC42 (Catalog #: C08-30G) at a ratio of 1:4 (PAK1:CDC42), in vitro. The PAK1 gene accession number is NM_002576.

Gene Aliases

PAKalpha, MGC130000, MGC130001

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

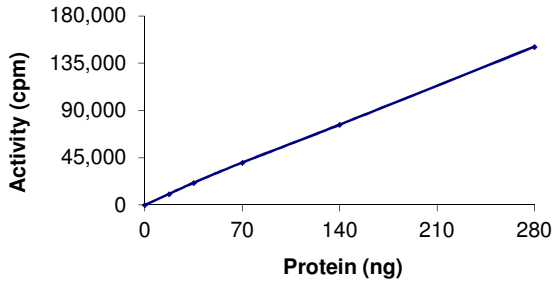
Scientific Background

PAK1 is a member of the p21-activated kinases (PAKs) which have been implicated in the regulation of cell morphology, motility and transformation. These serine/threonine kinases are activated by and are effectors of small GTPases, CDC42 and RAC. PAK1 belongs to the Group I PAKs which also includes PAK2 and PAK3 (1). PAK1 is a key regulator of the actin cytoskeleton, adhesion and cell motility. Inactive dimeric PAK1 is mainly cytosolic and interaction with the activators Cdc42-GTP and Rac1-GTP stimulates the kinase at the sites of cellular protrusions forming adhesions to the extracellular matrix (2).

References

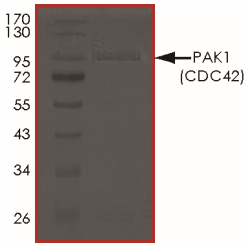
1. Jaffer, Z M. et al: p21-activated kinases: three more join the Pak. Int J Biochem Cell Biol. 2002 Jul;34(7):713-7.
2. Parrini, M C. et al: Spatiotemporal regulation of the Pak1 kinase. Biochem Soc Trans. 2005 Aug;33(Pt 4):646-8.

Specific Activity



The specific activity of PAK1/CDC42 was determined to be **43 nmol /min/mg** as per activity assay protocol.

Purity



The gel image is a representative of unactive PAK1 prior to activation with CDC42. The purity of PAK1 was determined to be **>80%** by densitometry, approx. MW **96kDa**.

PAK1/CDC42, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog #	P02-10G
Specific Activity	43 nmol/min/mg
Lot #	A1460-5
Purity	>80%
Concentration	0.1 µg/µl (PAK1); 0.4 µg/µl (CDC42)
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P02-10G)

Active PAK1/CDC42 (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PAK1/CDC42 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: P08-58)

PAKtide synthetic peptide substrate (RRRLSFAEPG) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active PAK1/CDC42, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active PAK1/CDC42 (Catalog #P02-10G)
- **Component 2.** 5μl $MnCl_2$ (12.5mM)/GTP (0.5mM) solution

Step 4. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 20μl and incubate the mixture in a water bath at 30°C for 20 minutes.

Step 5. After the 20 minute incubation period, add 5μl of 1mg/ml stock solution of substrate (Catalog #P08-58) to each assay vials, except of blank control which is replaced with an equal volume of distilled H_2O.

Step 6. After another 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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