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1-866-9 KINASE (54-6273)

PAK1/CDC42, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P02-10G-10

Lot # L206-4

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

Concentration

0.1 µg/µl

Formulation

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

Storage, Shipping 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.
Stability is 6 months at –70°C from date of shipment. Product shipped on dry ice.

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.

Purity

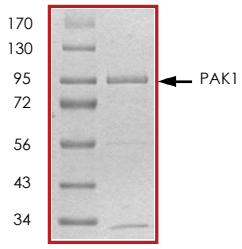
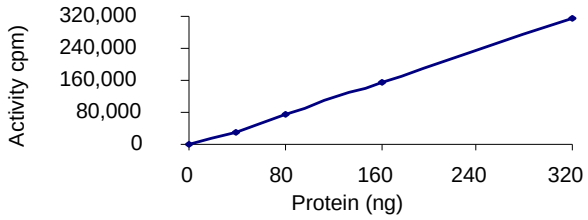


Figure 1. SDS-PAGE gel image

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**.

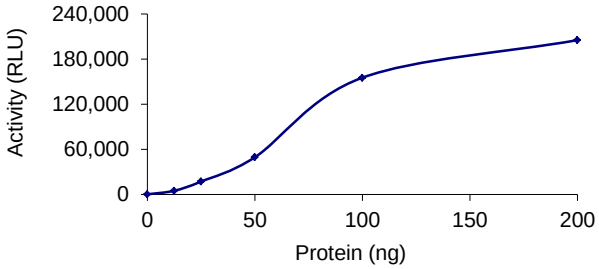
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of PAK1/CDC42 was determined to be **46 nmol /min/mg** as per activity assay protocol.
(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of PAK1/CDC42 was determined to be **32 nmol /min/mg** as per activity assay protocol.
(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

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 5μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 20μl and incubate the mixture in a water bath at 30°C for 15 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 20μl 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)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

PAK1/CDC42 Kinase Enzyme System (Promega, Catalog #:V4478)

PAK1/CDC42, Active, 10µg (0.1µg/µl)
PAKtide, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT (0.1M), 25µl
GTP Solution (5mM), 100µl

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10 mM (0.5ml)
ADP, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Reaction Buffer A (5X)
200mM Tris-HCl, pH 7. 5, 100mM $MgCl_2$ and 0.5 mg/ml BSA.

Assay Protocol

The PAK1/CDC42 assay is performed using the PAK1/CDC42 Kinase Enzyme System (Promega; Catalog #: V4478) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The PAK1/CDC42 reaction utilizes ATP and generates ADP. Then the ADP- Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding *the ADP-Glo™ Kinase Assay,* see the technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

Step 1. Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.

Step 2. Thaw the components of PAK1/CDC42 Enzyme System, ADP and ATP on ice.

Step 3. Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH_20.

Step 4. Prepare 250µll of GTP (0.5mM) solution by combining 25µl GTP (5mM) solution and 225µl of dH_20.

Step 5. Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH_20.

Step 6. Prepare diluted PAK1/CDC42 in 1X Buffer (diluted from 2X buffer) 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).

Step 7. In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1.	5µl of diluted Active PAK1/CDC42
Component 2.	5µl of GTP (0.5mM) solution
Component 3.	5µl of 2X Buffer

Step 8. Set up the blank control as outlined in step 7. You will replace the substrate with an equal volume of distilled H_2O at the step 10.

Step 9. Initiate the PAK1/CDC42 autophosphorylation by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 20µl and incubate the reaction mixture at 30°C for 15 minutes.

Step 10. After the 15 minutes of PAK1/CDC42 autophosphorylation, initiate the PAK1/CDC42 reactions by the addition of 5µl of substrate solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes. Add the distilled H_2O instead of substrate in the blank control.

Step 11. At the same time as the PAK1/CDC42 kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* technical Manual #TM313.

Step 12. Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.

Step 13. Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.

Step 14. Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).

Step 15. Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 7) and absence of substrate (Step 8 and 10) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)