3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

PAK1/CDC42, active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **K2893**
Storage Temperature –70 °C

Synonyms: PAKalpha, MGC130000, MGC130001

Product Description

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]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The gene accession number is NM 002576. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~96 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

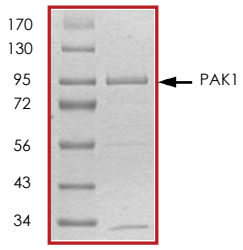
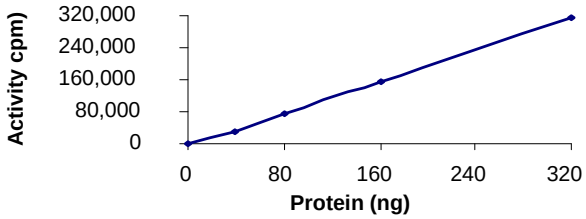


Figure 2.
Specific Activity of Typical Lot:
40–53 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.

Kinase Solution – Dilute the active PAK1/CDC42 (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration.
Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PAK1/CDC42 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at –20 °C.

γ-^{32}P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μl of 10 mM ATP Stock Solution, 100 μl of γ-^{32}P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (RRRLSFAEPG) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay
This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active PAK1/CDC42, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 15 μl:
   10 μl of Kinase Solution
   5 μl of 12.5 mM $MnCl_2$/0.5 mM GTP Solution
3. Initiate each reaction with the addition of 5 μl of the γ-^{32}P-ATP Assay Cocktail, bringing the final reaction volume to 20 μl. Incubate the mixture in a water bath at 30 °C for 20 minutes.
4. After the 20 minute incubation, add 5 μl of 1 mg/ml substrate solution to the reaction mixture.
5. Set up a blank control as outlined in steps 2–4, substituting 5 μl of cold water (4 °C) for the Substrate Solution.
6. After an additional 15 minute incubation, stop the reaction by spotting 20 μl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
7. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
8. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{32}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
9. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
10. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:
1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μl of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Jaffer, Z.M. et al., p21-activated kinases: three more join the Pak. Int. J. Biochem. Cell Biol., **34**, 713-717 (2002).
2. Parrini, M.C. et al., Spatiotemporal regulation of the Pak1 kinase. Biochem. Soc. Trans., **33**, 646-648 (2005).

AD,TD,MAM 02/17-1