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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P06-10G -05	5 µg
P06-10G -10	10 µg
P06-10G -20	20 µg

PAK6, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P06-10G
Lot # W320-4

Product Description

Recombinant full-length human PAK6 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_020168.

Gene Aliases

PAK5

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.

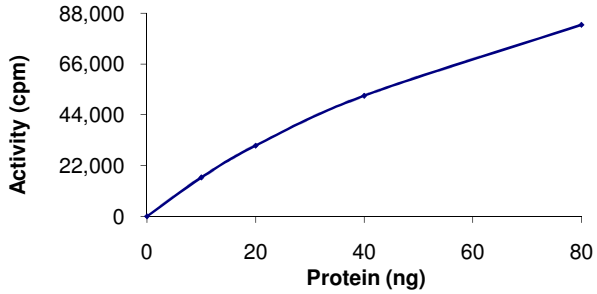
Scientific Background

PAK6 is a Ser/Thr protein kinase that is a member of the PAK (p21-activated kinase) family that are implicated in the regulation of a number of cellular processes, including cytoskeleton rearrangement, apoptosis and the MAP kinase signaling pathway. PAK6 is highly expressed in the testis and prostate tissues and interacts with the androgen receptor (AR). In response to androgen, PAK6 cotranslocate into the nucleus with AR (1). PAK6 is weakly expressed in normal prostate epithelium but the expression is increased in primary and metastatic prostate cancer cells and is further increased in tumors that relapse after androgen deprivation therapy (2).

References

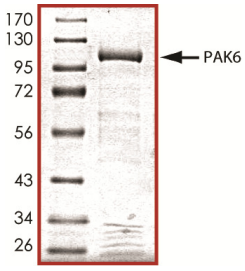
1. Yang F, et al: Androgen receptor specifically interacts with a novel p21-activated kinase, PAK6. *J. Biol. Chem.* 276: 15345-15353, 2001.
2. Kaur R, et al: Increased PAK6 expression in prostate cancer and identification of PAK6 associated proteins. Prostate. 2008 Oct 1;68(14):1510-6.

Specific Activity



The specific activity of PAK6 was determined to be **116 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>85%** by densitometry.
Approx. MW **110kDa**.

PAK6, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P06-10G
Specific Activity	116 nmol/min/mg
Specific Lot Number	W320-4
Purity	>85%
Concentration	0.1µg/µl
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 #: P06-10G)

Active PAK6 (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 PAK6 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 50 ng/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 PAK6, 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 PAK6 (Catalog #P06-10G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #P08-58)
- **Component 3.** 5µl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 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 250µM 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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