

PKN3/PRK3, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # P69-10G

Lot # B1976-3

Product Description

Recombinant full-length human PKN3/PRK3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_013355](#).

Gene Aliases

RP11-545E17.1

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.

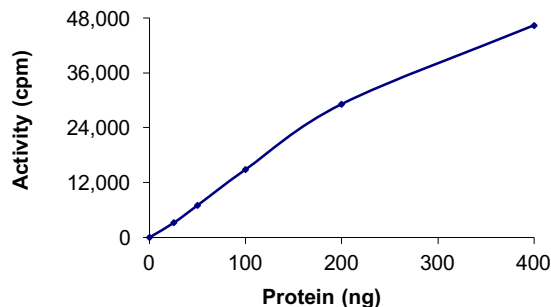
Scientific Background

PKN3 or PRK3 is a serine/threonine protein kinase which can associate with RHOA in a GTP-dependent manner. PKN3 can also interact with GRAF and GRAF2 via the SH3 domains and phosphorylate these proteins. PKN3 is a mediator of malignant growth of human prostate cancer cells and functions downstream of activated PI3K. PKN3 is upregulated in human tumor cells but not in surrounding nontumorigenic tissues. Inducible knockdown of PKN3 expression inhibited metastasis in a mouse prostate tumor model.

References

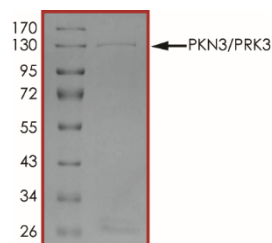
- Shibata, H. et al: PKN-beta interacts with the SH3 domains of Graf and a novel Graf related protein, Graf2, which are GTPase activating proteins for Rho family. *J. Biochem.* 130: 23-31, 2001.
- Leenders, F. et al: PKN3 is required for malignant prostate cell growth downstream of activated PI 3-kinase. *EMBO J.* 23: 3303-3313, 2004.

Specific Activity



The specific activity of PKN3/PRK3 was determined to be **3.5 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PKN3/PRK3 was determined to be **>70%** by densitometry, approx. MW **130 kDa**.

PKN3/PRK3, Active

Recombinant full-length protein expressed in Sf9 cells

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|--------------------|--|
| Catalog # | P69-10G |
| Specific Activity | 3.5 nmol/min/mg |
| Lot # | B1976-3 |
| Purity | >70% |
| Concentration | 0.05 µg/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 #: P69-10G)

Active PKN3/PRK3 (0.05µg/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 PKN3/PRK3 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 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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 #: S06-58)

RSK substrate (KRRRLSSLRA) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active PKN3/PRK3, 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 PKN3/PRK3 (Catalog # P69-10G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog # S06-58)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-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 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]/[(Reaction Volume) / (Spot Volume)]

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