

PKN1/PRK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # P70-11G

Lot # P1615-2

Product Description

Recombinant human PKN1/PRK1 (289-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The MSK2 gene accession number is [BC040061](#).

Gene Aliases

PRK1, DBK, PKN1, PKN, MGC46204, PAK1, PRKCL1, PKC-L1

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.

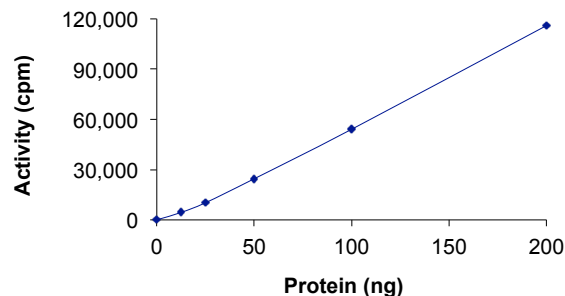
Scientific Background

PKN1/PRK1 belongs to the protein kinase C superfamily which is activated by Rho family of small G proteins. PKN1/PRK1 is known to mediate the Rho-dependent signaling pathway and it can be activated by phospholipids and by limited proteolysis (1). PDPK1/PDK may also mediate insulin signals to the actin cytoskeleton and the proteolytic activation of this kinase by caspase-3 or related proteases during apoptosis suggest its role in signal transduction related to apoptosis. PKN1/PRK signaling stimulates AR activity in the presence of adrenal androgens and in the presence of an AR antagonist (2).

References

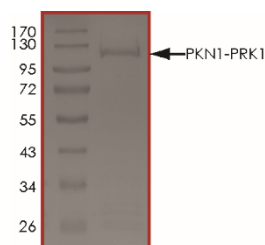
1. Amano, M.et.al: Identification of a putative target for rho as the serine-threonine kinase protein kinase N. Science 271: 648-651, 1996.
2. Metzger, E. et.al: A novel inducible transactivation domain in the androgen receptor: implications for PRK in prostate cancer. EMBO J. 22: 270-280, 2003.

Specific Activity



The specific activity of PKN1/PRK1 was determined to be **30 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PKN1/PRK1 was determined to be **>90%** by densitometry, approx. MW **115 kDa**.

PKN1/PRK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	P70-11G
Specific Activity	30 nmol/min/mg
Lot #	P1615-2
Purity	>90%
Concentration	0.1 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 #: P70-11G)

Active PKN1/PRK1 (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 PKN1/PRK1 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 #: C50-58)

CREBtide synthetic peptide substrate (KRREILSRPSYR) 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 PKN1/PRK1, 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 PKN1/PRK1 (Catalog # P70-11G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog # C50-58)
 - 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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