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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
P70-19G -05	5 µg
P70-19G -10	10 µg

ANXA4-PKN1(Aex2Pex13), Active

Recombinant human protein expressed in Sf9 cells

Catalog # P70-19G

Lot # M2634-11

Product Description

Recombinant human fusion protein ANXA4 (1-3a.a., exons1-2)-PKN1 (578a.a.-end, exons13-22) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number of ANXA4 is NM_001153, and the gene accession number of PKN1 is NM_002741.

Gene Aliases

ANX4; P32.5; PIG28; PP4-X; ZAP36; PAP-II; HEL-S-274; 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, and 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.

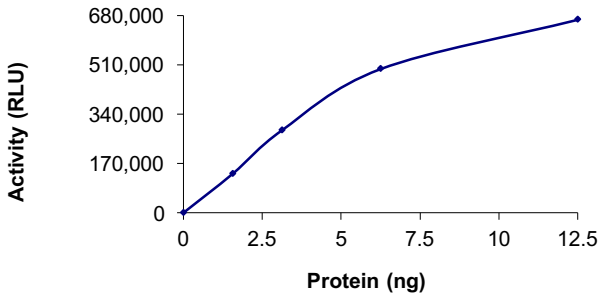
Scientific Background

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

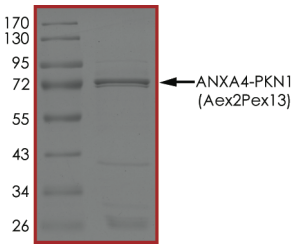
1. Amano, M.et.al: Identification of a putative target for rho as the serine-threonine kinase protein kinase N. Science 271: 648-651, 1996.
2. Metzger, E. et.al: A novel inducible transactivation domain in the androgen receptor: implications for PRK in prostate cancer. EMBO J. 22: 270-280, 2003.

Specific Activity



The specific activity of ANXA4-PKN1 (Aex2Pex13) was determined to be **228.9 nmol/min/mg** as per activity assay protocol.

Purity



The purity of ANXA4-PKN1 (Aex2Pex13) was determined to be **>85%** by densitometry, approx. MW **72 kDa**.

ANXA4-PKN1 (Aex2Pex13), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	P70-19G
Specific Activity	228.9 nmol/min/mg
Lot #	M2634-11
Purity	>85%
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 #: P70-19G)

Active ANXA4-PKN1 (Aex2Pex13) (0.1μg/μl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) 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 ANXA4-PKN1 (Aex2Pex13) for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50μM.

ADP-GloTM Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: C50-58)

CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled H2O to a final concentration of 0.2 mg/ml.

Assay Protocol

The ANXA4-PKN1 (Aex2Pex13) assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the ANXA4-PKN1 (Aex2Pex13) reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active ANXA4-PKN1 (Aex2Pex13), Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 μM example):

Component	Amount (μL)	Component	Amount (μL)
10μM ATP Solution	1	Substrate at 1mg/mL	80
Kinase Assay Buffer III (5x)	79		

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted ANXA4-PKN1 (Aex2Pex13) (Catalog # P70-19G).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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