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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P78-10BG-05	5 µg
P78-10BG-10	10 µg
P78-10BG-20	20 µg

PRKG1, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # P78-10BG
Lot # Q331-2B

Product Description

Recombinant full-length human PRKG1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006258.

Gene Aliases

PGK, CGKI, PRKG1B, PRKGR1B, FLJ36117, MGC71944, cGKI-BETA, cGKI-alpha, DKFZp686K042

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.

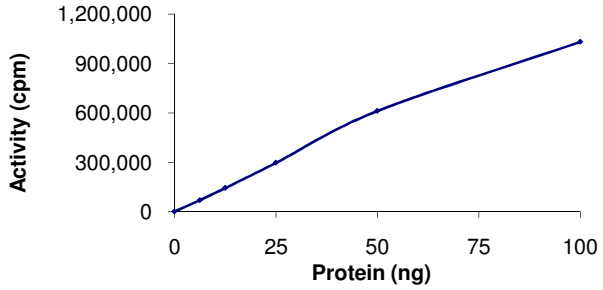
Scientific Background

PRKG1 is a homodimer, with each monomer containing a regulatory cGMP-binding domain and a catalytic domain (1). By Northern blot analysis PRKG1 was shown to be expressed at highest levels in bladder, uterus, adrenal gland, and fallopian tube. PRKG1 plays an important stimulatory role in platelet activation (2). Expression of recombinant PRKG1 in a reconstituted cell model enhanced von Willebrand factor-induced activation of the platelet integrin alpha-IIb/beta-3. Prkg1 knockout mice showed impaired platelet responses to VWF or low doses of thrombin and prolonged bleeding time. Human platelet aggregation induced by VWF or low-dose thrombin was inhibited by PRKG1 inhibitors but enhanced by cGMP.

References

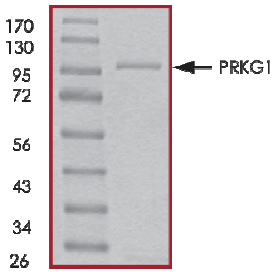
1. Orstavik, S. et al: Characterization of the human gene encoding the type I-alpha and type I-beta cGMP-dependent protein kinase (PRKG1). Genomics 42: 311-318, 1997.
2. Li, Z. et al: A stimulatory role for cGMP-dependent protein kinase in platelet activation. Cell 112: 77-86, 2003.

Specific Activity



The specific activity of PRKG1 was determined to be **780 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PRKG1 was determined to be **>95%** by densitometry, approx. MW **100kDa**.

PRKG1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P78-10BG
Specific Activity	780 nmol/min/mg
Specific Lot Number	Q331-2B
Purity	>95%
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 #: P78-10BG)

Active PRKG1 (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 PRKG1 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 final 50ng/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 #: S06-58)

RSK-sub peptide substrate (KRRRLSSLRA) 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 PRKG1, 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 PRKG1 (Catalog #P78-10BG)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #S06-58)
- **Component 3.** 2.5µl of 100µM cGMP solution (Catalog #G47-09)
- **Component 4.** 2.5µl 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 5 µl [^{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 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 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 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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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