

PKC β I, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P62-10G

Lot # K231-1

Product Description

Recombinant full-length human PKC β I was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [X06318](#).

Gene Aliases

PKCB; PRKCB; PRKCB2; MGC41878; PKC-beta

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

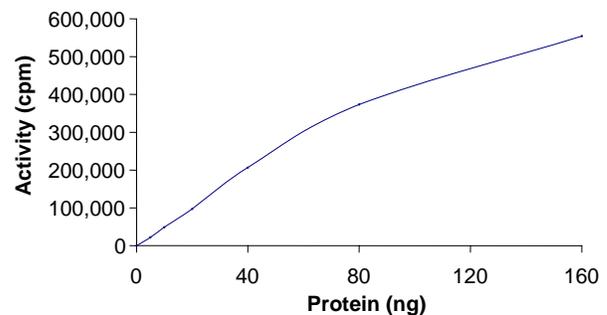
Scientific Background

PKC β I is a member of the PKC family (phospholipid-dependent serine/threonine kinase) and is highly related to PKC β II. Unlike the mature PKC β II mRNA and protein, which rapidly increase following acute insulin treatment, the PKC β I mRNA and protein levels remain unchanged (1). The stable overexpression of PKC β II, but not PKC β I, leads to insulin-stimulated glucose uptake into cells. Upon stimulation of B lymphocytes and mast cells, Syk regulates Btk, and Btk selectively regulates enzymatic activity of PKC β I. Specific regulation of PKC β I by Btk is consistent with the selective association of Btk with PKC β I (2).

References

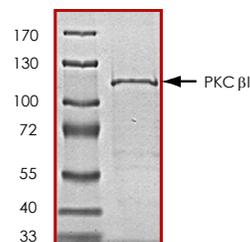
- Cooper, D R. et al: Ectopic expression of protein kinase C β 1, -delta, and -epsilon, but not -beta or -zeta, provide for insulin stimulation of glucose uptake in NIH-3T3 cells. Arch Biochem Biophys. 1999 Dec 1;372(1):69-79.
- Kawakami, Y. et al: Regulation of protein kinase C β 1 by two protein-tyrosine kinases, Btk and Syk. Proc Natl Acad Sci U S A. 2000 Jun 20;97(13):7423-8.

Specific Activity



The specific activity of PKC β I was determined to be **283 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **102kDa**.

PKC β I, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P62-10G
Specific Activity	283 nmol/min/mg
Specific Lot Number	K231-1
Purity	>90%
Concentration	0.1 $\mu\text{g}/\mu\text{l}$
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 #: P62-10G)

Active PKC β I (0.1 μ g/ μ l) diluted with Kinase Dilution Buffer I (Catalog #: K21-09) 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 PKC β I for optimal results).

Kinase Dilution Buffer I (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H₂O.

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.

[³²P]-ATP Assay Cocktail

Prepare 250 μ M [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³²P]-ATP (1mCi/100 μ l), 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 200 μ l aliquots at -20°C.

Substrate (Catalog #: P15-58)

PKCtide peptide substrate (ERM β PRKRQGSVRRRV) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PKC β I, 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 20 μ l:
 - Component 1. 10 μ l of diluted Active PKC β I (Catalog #P62-10G)
 - Component 2. 7.5 μ l of 1mg/ml stock solution of substrate (Catalog #P15-58)
 - Component 3. 2.5 μ l PKC lipid activator (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl₂). Sonicate lipid for 1 minute prior to use. (Catalog #L51-39)
- 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 μ l [³²P]-ATP Assay Cocktail bringing the final volume up to 25 μ l 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 μ l 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 [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³²P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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