PKC beta I (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP62

Lot No. K231-1
5 µg 0.1 µg/µl

Background:

PKC β I (PKCbetaI) 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).

Product Description:

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

Gene Aliases:

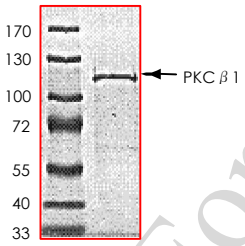
PKCB; PRKCB; PRKCB2; MGC41878; PKC-beta

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.

Purity & Molecular Weight:

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



Storage:

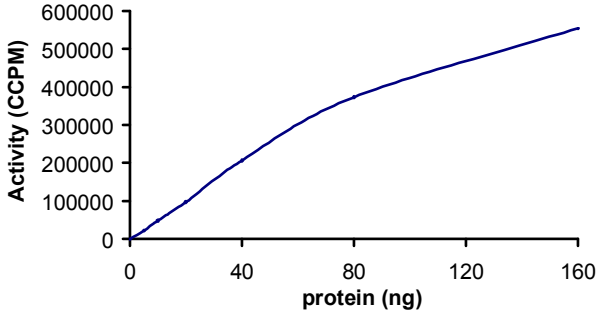
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at –70 °C, 1 year from date of shipment.

Specific Activity:

The specific activity was determined to be 283 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 7.5 µL of 1 mg/ml the Substrate Solution, 2.5 µL of lipid activator (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM $CaCl_2$), 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

PKC synthetic peptide substrate (ERMRPRKRQGSVRRRV) diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Cooper, D R. et al: Ectopic expression of protein kinase CbetaII, -delta, and -epsilon, but not -betaI or -zeta, provide for insulin stimulation of glucose uptake in NIH-3T3 cells. Arch Biochem Biophys. 1999 Dec 1;372(1):69-79.

2.Kawakami, Y. et al: Regulation of protein kinase CbetaI by two protein-tyrosine kinases, Btk and Syk. Proc Natl Acad Sci U S A. 2000 Jun 20;97(13):7423-8.

CycLex Co., Ltd
1063-103 Tera-Sawaoka, Ina, Nagano, Japan 396-0002
Fax: 81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp