PKC zeta (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP75

Lot No. B242-1
5 µg 0.1 µg/µl

Background:

PKC ζ (PKCzeta) is an atypical isoform of the PKC family. PKC ζ is found in both particulate and soluble fractions and cannot be activated by phorbol ester. Overexpression of PKC ζ and subsequent phorbol ester treatment abolished phorbol ester-induced reduction in cell proliferation (1). Overexpression of PKC ζ also potentiates phorbol ester-induced mitogen-activated protein (MAP) kinase activation in a PKC-dependent manner. PKC ζ is an upstream modulator of p70S6K, an important regulator of cell proliferation (2).

Product Description:

Recombinant full-length human PKC ζ was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_002744.

Gene Aliases:

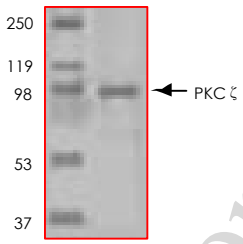
PRKCZ; PRKCZ

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 93kDa.



Storage:

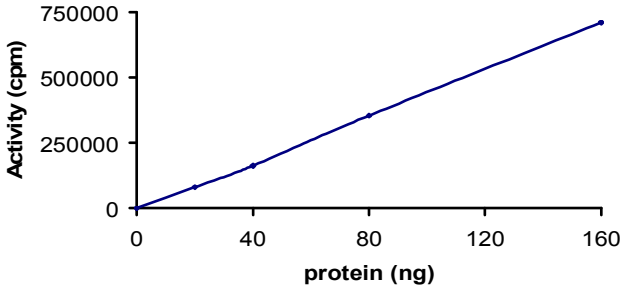
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 114 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, 10 µL of 1 mg/ml the Substrate Solution, 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:

CREBtide synthetic peptide substrate (KRREILSRRPSYR) 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 $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Kim, SJ. Et al: Phorbol ester effects in atypical protein kinase C zeta overexpressing NIH3T3 cells: possible evidence for crosstalk between protein kinase C isoforms. Biochem Biophys Res Commun. 1997 Aug 18;237(2):336-9.

2.Romanelli, A. et al: p70 S6 kinase is regulated by protein kinase Czeta and participates in a phosphoinositide 3-kinase-regulated signalling complex. Mol Cell Biol. 1999 Apr;19(4):2921-8.

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