BioVision
BioVision Incorporated
155 South Milpitas Boulevard, Milpitas, CA 95035 USA Phone: (408)493-1800 Fax: (408)493-1801

Product Specification

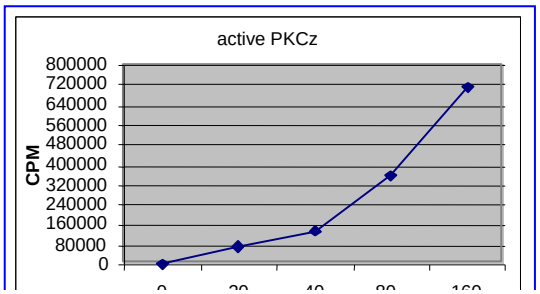
PKCzeta, active
(Full-length recombinant protein expressed in Sf 9 cells)

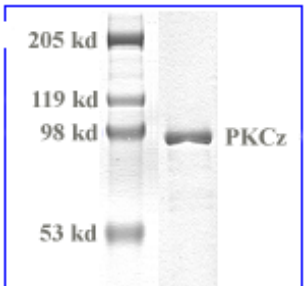
Catalog #: 7718
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 93 nmol/min/mg

Quality Control Analysis
Activity assessment
PKC zeta protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate to phosphorylate the CREBtide in the following assay condition:

10 µl diluted PKC z protein
5 µl CREBtide (1 mg/ml stock)
5 µl water
5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.





Purity assessment
1 µg of PKCz protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKC zeta product, and the band was at ~93 kDa (Fig. 2).

Product Description
Recombinant full length human PKC zeta containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_002744.
This material is sold for research purposes only.

Specific Activity
93 nmol phosphate incorporated into CREBtide per minute per mg protein at 30^0C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation
Recombinant proteins in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 30% glycerol).

Storage and Stability
Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background
PKCz (PKC zeta) is an atypical isoform of the PKC family. PKCz is found in both particulate and soluble fractions and cannot be activated by phorbol ester. Treatment of cells with phorbol ester which activates PKCalpha, gamma, delta, and epsilon isoforms in NIH3T3 cells significantly reduced proliferation of cells. Overexpression of PKCz and subsequent phorbol ester treatment abolished phorbol ester-induced reduction in cell proliferation (1). Overexpression of PKCz also potentiated phorbol ester-induced mitogen-activated protein (MAP) kinase activation in a PKC-dependent manner. The effects of PKCz overexpression on proliferation and MAP kinase activation are proportional to the levels of PKCz expression.
PKCz as an upstream modulator of p70S6K, an important regulator of cell proliferation (2). Kinase-inactive PKCz mutant antagonized activation of p70S6K by epidermal growth factor, PDK-1, and activated Cdc42 and PI3-K. Overexpression of a constitutively active PKCz mutant (myristoylated PKCzeta [myr-PKCzeta]) only modestly activated p70S6K but this mutant cooperated with PDK-1 for the activation of p70S6K. PDK-1-induced activation of a C-terminal truncation mutant of p70S6K was also enhanced by myr-PKCz. p70S6K can associate with both PDK-1 and PKCz in vivo in a growth factor-independent manner, while PDK-1 and PKCz can also associate with each other, suggesting the existence of a multimeric PI3-K signalling complex.

References
1. Kim SJ, Chang YY, Kang SS, Chun JS. 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, Martin KA, Toker A, Blenis J. 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.