

Product Specification

PKC delta, active

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

Catalog # 7739-5

Lot # _____

Size: 5 µg protein in 50 µl

Specific activity: 302 nmol/min/mg

Quality Control Analysis

Activity assessment

PKC delta protein (~100 ng/µl concentration) was diluted to 26.6ng/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 MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the CREBtide (KRREILSRRPSYR) in the following assay condition:

7.5 µl diluted PKC delta protein

10 µl CREBtide (1 mg/ml stock)

2.5 µl lipid activators (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, 25 mM beta-glycerophosphate, 1 mM sodium orthovanadate, 1 mM dithioreitol, 1 mM CaCl₂). Sonicate for 1 minute prior to use.

5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] 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.

Fig. 1 PKC delta activity assay

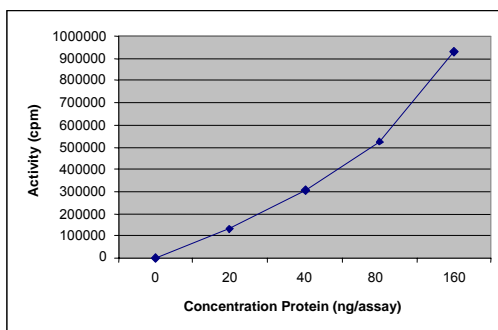
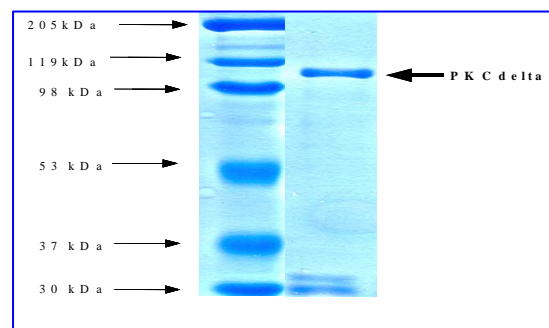


Fig. 2 PKC delta protein gel



Purity assessment

1 µg of PKC delta protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >80% purity of the PKC delta product, and the band was at ~104 kDa (Fig. 2).

Product Description

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

The gene accession number is NM_006254.

This material is sold for research purposes only.

Specific Activity

302 nmol phosphate incorporated into CREBtide substrate per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μ M ATP (0.83 μ Ci/assay).

Formulation

Recombinant protein 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, 25% 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

Protein kinase C delta (PKC delta) is a member of the protein kinase C (PKC) family of serine-threonine kinases. It is a 79 kd protein kinase that shows strict dependence on the presence of phospholipids, but shows no activation by Ca²⁺ (1). Phosphatidylinositol is the most potent activator of PKC delta. Apparent kinetic constants for synthetic oligopeptides (MBP4-14, EGFR peptide and epsilon-peptide) are quite different between PKC delta and other PKCs. Northern blot analysis indicated that PKC delta is widely distributed in almost all the tissues and is a major isoform of PKC expressed in hemopoietic cells (2). PKC delta is involved in fundamental cellular functions regulated by diacylglycerols and mimicked by phorbol esters. PKC delta is partially associated with the insoluble fraction in cells even in the absence of phorbol 12-myristate 13-acetate (PMA). Upon PMA stimulation, both it translocate to the insoluble fraction of cell homogenates (3). Overexpression of PKC-delta induces significant changes in morphology and causes the cells to grow more slowly and to a decreased cell density in confluent cultures. These changes are accentuated by treatment with PMA. None of the PKC-delta overexpressers grow in soft agar with or without PMA.

References

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2. Mischak H, Bodenteich A, Kolch W, Goodnight J, Hofer F, Mushinski JF. Mouse protein kinase C-delta, the major isoform expressed in mouse hemopoietic cells: sequence of the cDNA, expression patterns, and characterization of the protein. *Biochemistry.* 1991 Aug 13;30(32):7925-31.
3. Mischak H, Goodnight JA, Kolch W, Martiny-Baron G, Schaehtle C, Kazanietz MG, Blumberg PM, Pierce JH, Mushinski JF. Overexpression of protein kinase C-delta and -epsilon in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. *J Biol Chem.* 1993 Mar 25;268(9):6090-6.