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Product Specification

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

Catalog #: 7745-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 658 nmol/min/mg

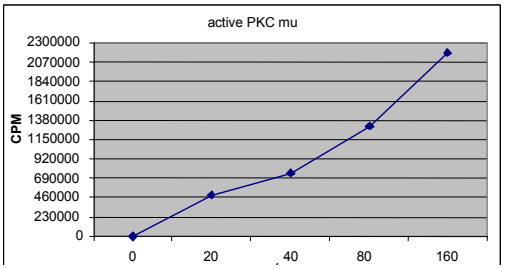
Quality Control Analysis

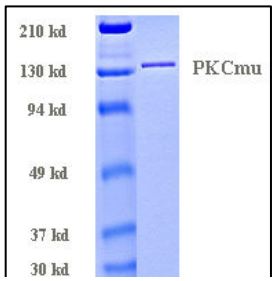
Activity assessment

PKC mu 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 the CREBTIDE substrate peptide (KRREILSRRPSYR) in the following assay condition:

10 µl diluted PKC mu protein
10 µl CREBTIDE substrate peptide (1 mg/ml stock)
5 µl [^{32}P] ATP mixture (250 µM ATP, 166 nCi/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 PKC mu protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKCmu product, and the band was ae ~131 kDa (Fig. 2).

Product Description
Recombinant full length human PKC mu containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is X75756.
This material is sold for research purposes only.

Specific Activity
680 nmol phosphate incorporated into CREBTIDE substrate peptide per minute per mg protein at 30˚C 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, 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 mu (PKC mu) is a novel member of the protein kinase C (PKC) family that differs from the other isoenzymes in structural and enzymatic properties. It is characterized by the presence of a pleckstrin homology (PH) domain and an amino-terminal hydrophobic region and has substrate specificity distinct from other PKC isoforms. PKCmu is a ubiquitous PKC isotype with the highest expression in the thymus, lung and peripheral blood mononuclear cells (1). PKC mu forms a complex in vivo with a phosphatidylinositol 4-kinase and a phosphatidylinositol-4-phosphate 5-kinase. A region of PKC mu between the amino-terminal transmembrane domain and the pleckstrin homology domain is shown to be involved in the association with the lipid kinases (2). PKC mu was also shown to associate with the B cell receptor (BCR) complex and its activity is up-regulated after cross-linking the BCR and CD19 on B cells (3). PKC mu co-precipitates with Syk and phospholipase C-gamma 1/2 (PLC gamma 1/2) and in vitro phosphorylation of fusion proteins showed that both Syk and PLC gamma 1 are potential substrates of PKC mu in vivo. In addition, specific interaction of PKC mu and 14-3-3tau can be shown in the T cell line Jurkat by immunocoprecipitiation and by pulldown assays (4). 14-3-3tau is not a substrate of PKC mu and strongly down-regulates PKC mu kinase activity in vitro. In response to various stimuli, PKC mu activates the mitogen-activated protein kinase (p42/ERK1 MAPK cascade) but does not affect the related c-jun N-terminal kinase or p38 MAPK (5).

References
1. Rennecke J, Johannes FJ, Richter KH, Kittstein W, Marks F, Gschwendt M. *Immunological demonstration of protein kinase C mu in murine tissues and various cell lines. Differential recognition of phosphorylated forms and lack of down-regulation upon 12-O-tetradecanoylphorphol-13-acetate treatment of cells.* Eur J Biochem. 1996 Dec 1;242(2):428-32.
2. Nishikawa K, Toker A, Wong K, Marignani PA, Johannes FJ, Cantley LC. *Association of protein kinase Cmu with type II phosphatidylinositol 4-kinase and type I phosphatidylinositol-4-phosphate 5-kinase.* J Biol Chem. 1998 Sep 4;273(36):23126-33.
3. Sidorenko SP, Law CL, Klaus SJ, Chandran KA, Takata M, Kurosaki T, Clark EA. *Protein kinase C mu (PKC mu) associates with the B cell antigen receptor complex and regulates lymphocyte signaling.* Immunity. 1996 Oct;5(4):353-63.
4. Hausser A, Storz P, Link G, Stoll H, Liu YC, Altman A, Pfizenmaier K, Johannes FJ. *Protein kinase C*

mu selectively activates the mitogen-activated protein kinase (MAPK) p42 pathway. FEBS Lett. 2001 Mar 9;492(1-2):39-44.