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Product Specification

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

Catalog #: 7731-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 86 nmol/min/mg

Quality Control Analysis
Activity assessment
PKC eta protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (5 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 peptide substrate (ERMRPRKRQGSVRRRV) diluted in distilled water to 1 mg/ml. Assay conditions:

1) 10 µl diluted PKC eta protein
2) 7.5 µl peptide substrate (ERMRPRKRQGSVRRRV) (1 mg/ml stock)
3) 2.5 µl lipid activator (0.5 mg/ml phosphatidylserine, 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM $CaCl_2$);sonicate lipid 1 mnute prior to use.
4) 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.

Specific Activity

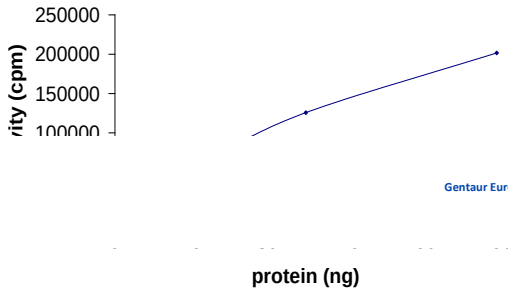

Fig. 1. The specific activity of PKCeta was determined to be **86 nmol /min/mg** as per activity assay protocol.

Purity

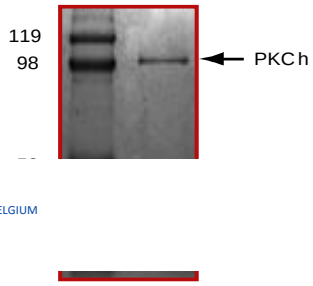

Fig. 2 The purity was determined to be **>90%** by densitometry. Approx. MW **103 kDa**.

Purity assessment
1 µg of PKC eta protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKC eta product, and the band was at ~103 kDa (Fig. 2).

Product Description
Recombinant full length human PKC eta containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_006255.

Specific Activity
86 nmol phosphate incorporated into peptide substrate (ERMRPRKRQGSVRRRV) 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
PKC eta is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can phosphorylate a wide variety of protein targets known to be involved in diverse cellular signaling pathways. PKC eta is predominantly expressed in squamous cell epithelia and induces terminal differentiation of keratinocytes. PKC eta that is endogenously expressed or overexpressed is found to associate with the cyclin E/cdk2/p21 complex in keratinocytes of mice and humans (1).

References
1. Kashiwagi, M. et al: PKCeta associates with cyclin E/cdk2/p21 complex, phosphorylates p21 and inhibits cdk2 kinase in keratinocytes. Oncogene. 2000 Dec 14;19(54):6334-41.

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