

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 MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the peptide substrate (ERM₂RPRKRQGSVRRRV) diluted in distilled water to 1 mg/ml. Assay conditions:

- 1) 10 µl diluted PKC eta protein
- 2) 7.5 µl peptide substrate (ERM₂RPRKRQGSVRRRV) (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 CaCl₂);sonicate lipid 1 mminute prior to use.
- 4) 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 30⁰C 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.

Specific Activity

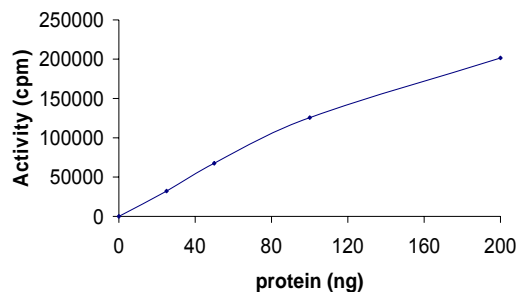


Fig. 1 The specific activity of PKCeta was determined to be **86 nmol /min/mg** as per activity assay protocol.

Purity

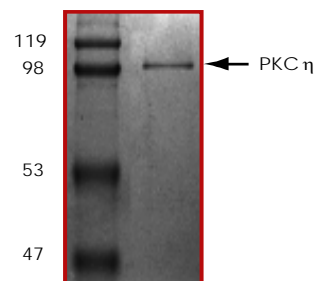


Fig. 2 The purity was determined to be **>90%** by densitometry. Approx. MW **103kDa**.

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 (ERM₂PRKRQGSVRRRV) 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.