

## Product Specification

### PKCε, active

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

Catalog #: 7753-5  
 Lot # \_\_\_\_\_  
 Aliquot size: 5 µg protein in 50 µl  
 Specific activity: 1258 nmol/min/mg

### Quality Control Analysis

#### Activity assessment

PKCepsilon protein (~100 ng/µl concentration) was diluted to 12.5ng/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<sub>2</sub>, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the PKCε substrate peptide (ERM<sub>1</sub>PRKRQGSVRRRV) in the following assay condition:

- 10 µl diluted PKCepsilon protein
- 7.5 µl PKCε substrate peptide (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 dithioereitol, 1 mM CaCl<sub>2</sub>). Sonicate for 1 minute prior to use.
- 5 µl [<sup>32</sup>P] ATP mixture (250 µM ATP, 166 nCi/µl in 4x assay dilution buffer)

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

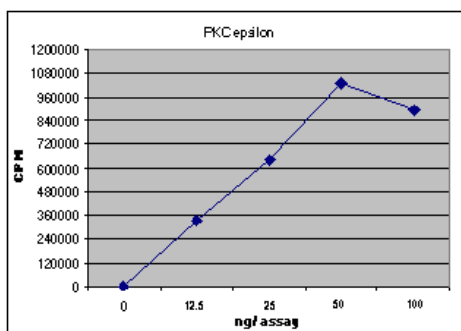


Fig. 1 PKCepsilon activity assay

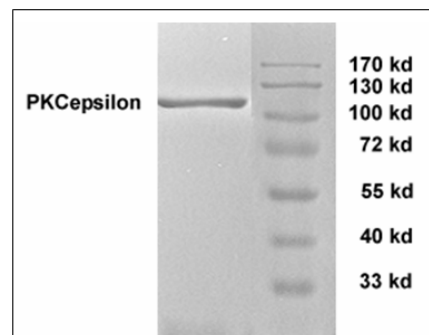


Fig. 2 PKCepsilon protein gel

#### Purity assessment

1.5 µg of PKCepsilon protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKCepsilon product, and the band was at ~110 kDa (Fig. 2).

## **Product Description**

Recombinant full length human PKCepsilon containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM\_005400.

This material is sold for research purposes only.

## Specific Activity

1258 nmol phosphate incorporated into PKCε 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 (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. Protein kinase C epsilon (PRKCE), one of the PKC family members, has been shown to be involved in many different cellular functions, such as neuron channel activation (1), apoptosis (2), cardioprotection from ischemia (3, 4), heat shock response, as well as insulin exocytosis. Knockout studies in mice suggest that this kinase is important for lipopolysaccharide (LPS)-mediated signaling in activated macrophages and may also play a role in controlling anxiety-like behavior (5).

## Reference

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