

PKC epsilon (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP65

Lot No. L068-1
5 µg 0.1 µg/µl

Background:

PKC ϵ (PKCepsilon) 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 ϵ is involved in many different cellular functions, such as neuron channel activation, cardioprotection from ischemia (1), 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 control anxiety-like behavior (2).

Product Description:

Recombinant full-length human PKC ϵ was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_005400.

Gene Aliases:

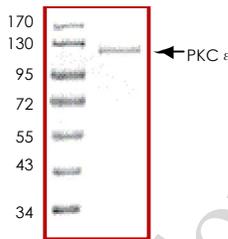
PRKCE

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 110kDa.



Storage:

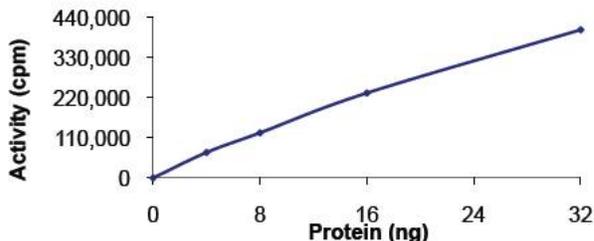
Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at -70°C , 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 1050 nmol /min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25 μ L reaction consisting of 5 μ L of 5 X Kinase Assay Buffer, 7.5 μ L of 1 mg/ml the Substrate Solution, 2.5 μ L of lipid activator (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl_2), 5 μ L of diluted kinase and 5 μ L of 250 μ M ATP solution containing [γ ^{32}P] ATP (0.167 μ Ci/ μ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 μ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

PKC synthetic peptide substrate (ERM RPRKRQGSVRRRV) diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β -glycerol-phosphate, 25mM MgCl_2 , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

- 1.Chen, C H. et al: Cardioprotection from ischemia by a brief exposure to physiological levels of ethanol: role of epsilon protein kinase C. Proc. Nat. Acad. Sci. 96: 12784-12789, 1999.
- 2.Hodge, C W. et al: Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase C-epsilon. J. Clin. Invest. 110: 1003-1010, 2002.

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