

PKC eta (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP67

Lot No. B034-1
5 µg 0.1 µg/µl

Background:

PKC η (PKCeta) 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 η is predominantly expressed in squamous cell epithelia and induces terminal differentiation of keratinocytes. PKC η that is endogenously expressed or overexpressed is found to associate with the cyclin E/cdk2/p21 complex in keratinocytes of mice and humans (1).

Product Description:

Recombinant full-length human PKC η was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_006255.

Gene Aliases:

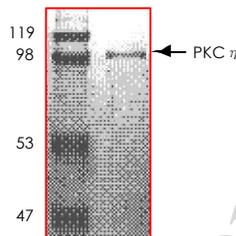
PKCL; PKC-L; PRKCL; MGC5363; PRKCH

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 103kDa.



Storage:

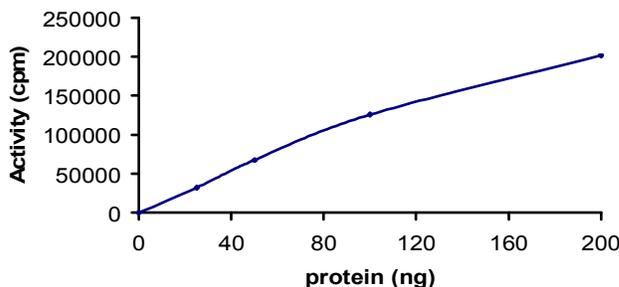
Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

**Specific Activity:**

The specific activity was determined to be 86 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 (ERM₂PRK₂RQGSVRRRV) diluted in distilled H₂O 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.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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