PKC iota (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP68

Lot No. A252-1
5 µg 0.1 µg/µl

Background:

PKC ι (PKCiota) is a member of the protein kinase C family of serine-threonine kinases. The amino acid sequence of PKC ι showed greatest homology to PKCζ (PKCzeta), with 72% identity overall rising to 84% in the catalytic domain. PKC ι has been implicated in Ras signaling and is a critical downstream effector of oncogenic Ras in the colonic epithelium. Transgenic mice expressing constitutively active PKC ι in the colon are highly susceptible to carcinogen-induced colon carcinogenesis (1).

Product Description:

Recombinant full-length human PKC ι was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_002740.

Gene Aliases:

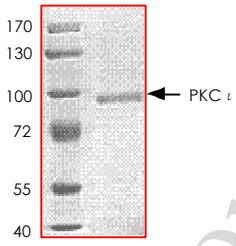
PRKCI; DXS1179E

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 98kDa.



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:

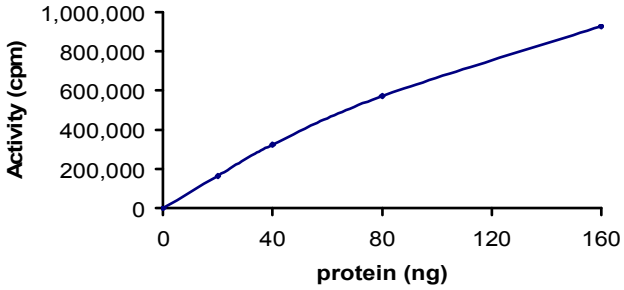
Unopened vial at –70 °C, 1 year from date of shipment.

For Reference Purpose Only!

For Research Use Only, Not for use in diagnostic procedures

Specific Activity:

The specific activity was determined to be 664 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 7.5 µL of 1 mg/ml the Substrate Solution, 2.5 µL 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 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL 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:

CREBtide synthetic peptide substrate (KRREILSRRPSYR) 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.Murray, N R. et al: Protein kinase Ciota is required for Ras transformation and colon carcinogenesis in vivo. J Cell Biol. 2004 Mar 15;164(6):797-802.

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