

## Product: PKC $\iota$ , Active

Catalog #: 02-2048

Amount: 5  $\mu$ g

### Product Description

Recombinant full-length human PKC iota 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.

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . 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.

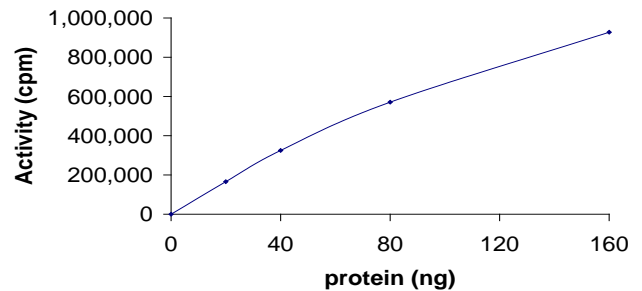
### Scientific Background

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

### References

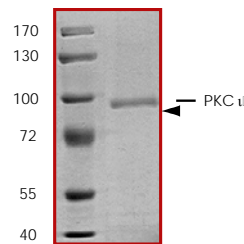
- Murray, N R. et al: Protein kinase Ciota is required for Ras transformation and colon carcinogenesis in vivo. J Cell Biol. 2004 Mar 15;164(6):797-802.

### Specific Activity



The specific activity of PKCiota was determined to be **664 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **98kDa**.

## PKC $\iota$ , Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number **02-2048**

Quantity **5 $\mu$ g**

Specific Activity **664 nmol/min/mg**

Specific Lot Number **A252-1**

Purity **>90%**  
 Format **5 $\mu$ g in 50 $\mu$ l**  
 Concentration **0.1 $\mu$ g/ $\mu$ l**  
 Stability **1yr At  $-70^{\circ}\text{C}$  from date of shipment**  
 Storage & Shipping **Store product at  $-70^{\circ}\text{C}$ . 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. Product shipped on dry ice.**

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## Activity Assay Protocol

### Reaction Components

#### Active Kinase (Catalog #: 02-2048)

Active PKCiota (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PKCiota for optimal results).

#### Kinase Dilution Buffer, pH 7.2 (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled .

#### Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

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

#### [γ]-ATP Assay Cocktail

Prepare 250µM [γ]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>32</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

#### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at -20°C.

#### Substrate

CREBtide synthetic peptide substrate (KRREILSRPSYR) diluted in distilled to a final concentration of 1mg/ml.

### Assay Protocol

- Step 1. Thaw [<sup>32</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PKCiota, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1. 10µl of diluted Active PKCiota (Catalog # P68-10G-20).
  - Component 2. 7.5µl of 1mg/ml stock solution of substrate
  - Component 3. 2.5µl lipid activator (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl<sub>2</sub>). Sonicate lipid for 1 minute prior to use.
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5µl [<sup>32</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

#### Calculation of [P<sup>32</sup>]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>32</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

#### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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