

## PKC alpha (Xenopus), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP61

Lot No. K111-1  
5 µg 0.1 µg/µl

### Background:

PKC $\alpha$  (PKC-alpha) is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC $\alpha$  has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control (1). PKC $\alpha$  has been assigned to the chromosome region 17q22-q23.2 and has been identified as a fundamental regulator of cardiac contractility and Ca(2+) handling in myocytes (2).

### Product Description:

Recombinant full-length xenopus PKC $\alpha$  was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is BC078065.

### Gene Aliases:

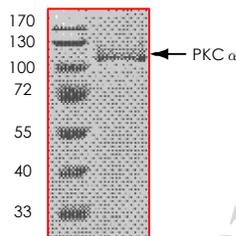
PRKCA

### 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 >80% by densitometry. Approx. MW 103kDa.



### Storage:

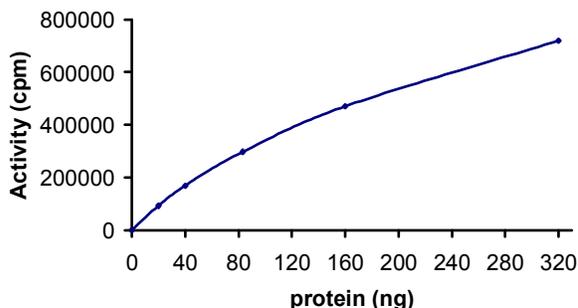
Store product at  $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Stability:

Unopened vial at  $-70^{\circ}\text{C}$ , 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 219 nmol/min/mg as per Activity Assay Protocol.



**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 7.5  $\mu$ L of 1 mg/ml the Substrate Solution, 2.5  $\mu$ 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  $\text{CaCl}_2$ ), 5  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}\text{P}$ ] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ 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:**

CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled  $\text{H}_2\text{O}$  to a final concentration of 1 mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, 12.5mM  $\beta$ -glycerol-phosphate, 25mM  $\text{MgCl}_2$ , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**References:**

1. Coussens, L. et al: Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233: 859-866, 1986.
2. Braz, J C. et al: PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nature Med.* 10: 248-254, 2004.

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