

## PKC $\alpha$ , Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P61-18G

Lot # Q2258-7

### Product Description

Recombinant full-length human PKC alpha was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_002737](#).

### Gene Aliases

AAG6, PKCA, PRKCA, PRKACA, MGC129900, MGC129901

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

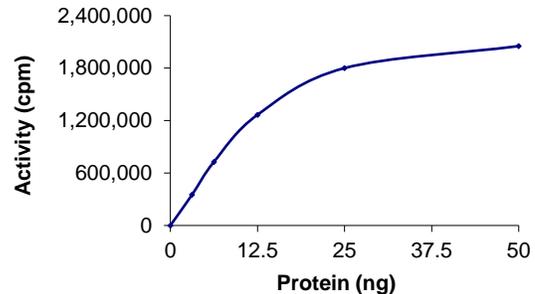
### Scientific Background

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).

### References

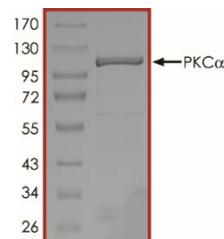
1. Coussens, L. et al: Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233: 859-866, 1986.
2. Braz, J C. et al: PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nature Med.* 10: 248-254, 2004.

### Specific Activity



The specific activity of PKC $\alpha$  was determined to be **3,200 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of PKC $\alpha$  was determined to be **>95%** by densitometry. Approx. MW **103kDa**.

## PKC $\alpha$ , Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	P61-18G
Specific Activity	3,200 nmol/min/mg
Lot #	Q2258-7
Purity	>95%
Concentration	0.1 µg/µl
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 #: P61-18G)

Active PKC $\alpha$  (0.1 $\mu$ g/ $\mu$ l) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) 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 PKC $\alpha$  for optimal results).

### Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/ $\mu$ l BSA and 5% glycerol solution.

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

Buffer components: 25mM MOPS, pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250 $\mu$ M [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 $\mu$ l [<sup>33</sup>P]-ATP (1mCi/100 $\mu$ l), 5.75ml of Kinase Assay Buffer I (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 I (Catalog #: K01-09). Store 200 $\mu$ l aliquots at -20°C.

### Substrate (Catalog #: C50-58)

CREBtide synthetic peptide substrate (KRREILSRPPSYR) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1. Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PKC $\alpha$ , Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 $\mu$ l:
  - Component 1. 10 $\mu$ l of diluted Active PKC $\alpha$  (Catalog #P61-18G)
  - Component 2. 5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog#C50-58)
  - Component 3. 2.5 $\mu$ l of PKC lipid activator (Catalog # L51-39). Sonicate lipid for 1 minute prior to use
  - Component 4. 2.5 $\mu$ l of distilled H<sub>2</sub>O (4°C)
- 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 5  $\mu$ l [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l 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 20  $\mu$ l 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5  $\mu$ l [<sup>33</sup>P]-ATP / pmoles of ATP (in 5  $\mu$ l of a 250  $\mu$ M ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]

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