

## PKC $\gamma$ , Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P66-10G-10

Lot # R002-1

### Product Description

Recombinant full-length human PKC $\gamma$  was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_002739](#).

### Gene Aliases

PKCC; PKCG; SCA14; MGC57564; PKC-gamma; PRKCG

### Concentration

0.1 $\mu$ g/ $\mu$ l

### 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, Shipping 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. Stability is 1yr at  $-70^{\circ}\text{C}$  from date of shipment. Product shipped on dry ice.

### Scientific Background

PKC $\gamma$  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. In the brain, PKC $\gamma$  is translocated to cell membranes during ischemia and is rapidly removed or degraded during the second otherwise lethal ischemic insult in preconditioned brains. This suggests that ischemic preconditioning enhances downregulation of cell signaling mediated by PKC $\gamma$  and may thereby provide neuroprotection (1).

### References

1. Shamloo, M. et al: Rapid decline in protein kinase C $\gamma$  levels in the synaptosomal fraction of rat hippocampus after ischemic preconditioning. Neuroreport. 1999 Apr 6;10(5):931-5.

### Purity

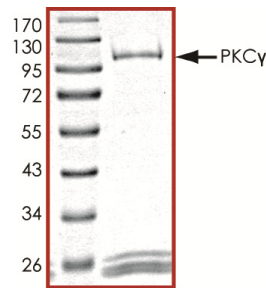
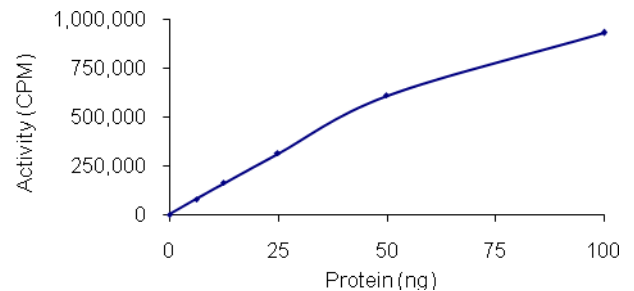


Figure 1. SDS-PAGE gel image

The purity of PKC $\gamma$  was determined to be **>80%** by densitometry, approx. MW **105kDa**.

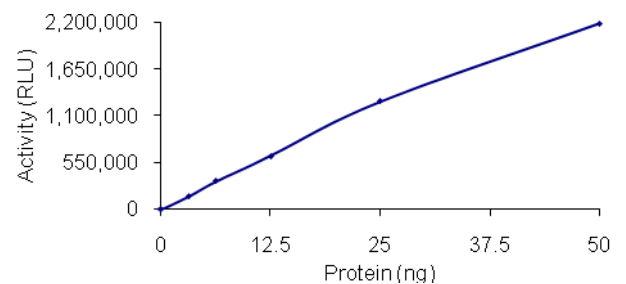
### Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of PKC $\gamma$  was determined to be **589nmol/min/mg** as per activity assay protocol. (For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP- Glo™ Assay Data



The specific activity of PKC $\gamma$  was determined to be **929nmol/min/mg** as per activity assay protocol. (For ADP- Glo™ Assay Protocol on this product please see pg. 3)

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: P66-10G-10)

Active PKC $\gamma$  (0.1 $\mu$ g/ $\mu$ l) diluted with Kinase Dilution Buffer I (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 PKC $\gamma$  for optimal results).

### Kinase Dilution Buffer I (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H<sub>2</sub>O.

### 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 #: P15-58)

PKCtide peptide substrate (ERM $\overline{R}$ PRKRQGSVRRRV) 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 $\gamma$ , 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 $\gamma$  (Catalog #P66-10G-10)
  - Component 2.** 7.5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog #P15-58)
  - Component 3.** 2.5 $\mu$ l PKC lipid activator (0.5 mg/ml phosphatidylserine and 0.05mg/ml diacylglycerol in 20mM MOPS, pH 7.2, containing 1mM CaCl<sub>2</sub>). Sonicate or vortex lipid for 1 minute prior to use. (Catalog #L51-39)
- 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 (cpm) 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)]

# ADP-Glo™ Activity Assay Protocol

## Reaction Components

### PKC $\gamma$ Kinase Enzyme System (Promega, Catalog #:V3391)

PKC $\gamma$ , Active, 10 $\mu$ g (0.1 $\mu$ g/ $\mu$ l)  
PKCtide, substrate, 1ml (1mg/ml)  
Reaction Buffer A (5X), 1.5ml  
DTT solution (0.1M), 25 $\mu$ l  
PKC Lipid Activator (10X), 500 $\mu$ l

### ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10mM (0.5ml)  
ADP, 10mM (0.5ml)  
ADP-Glo™ Reagent (5ml)  
Kinase Detection Buffer (10ml)  
Kinase Detection Substrate (Lyophilized)

### Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl<sub>2</sub> and 0.5 mg/ml BSA.

## Assay Protocol

The PKC $\gamma$  assay is performed using the PKC $\gamma$  Kinase Enzyme System (Promega; Catalog #: V3391) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The PKC $\gamma$  reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html).

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of PKC $\gamma$  Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400 $\mu$ l Reaction Buffer A, 1 $\mu$ l DTT and 599 $\mu$ l of dH<sub>2</sub>O.
- Step 4.** Prepare 1ml of 250 $\mu$ M ATP Assay Solution by adding 25 $\mu$ l ATP solution (10mM) to 500 $\mu$ l of 2X Buffer and 475 $\mu$ l of dH<sub>2</sub>O.
- Step 5.** Prepare diluted PKC $\gamma$  in 1X Buffer (diluted from 2X buffer) 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 $\gamma$  for optimal results).
- Step 6.** In a white 96-well plate (Corning Cat.# 3912), add the following reaction components bringing the initial reaction volume up to 20 $\mu$ l:

<b>Component 1.</b>	5 $\mu$ l of diluted Active PKC $\gamma$
<b>Component 2.</b>	5 $\mu$ l of 1mg/ml stock solution of substrate
<b>Component 3.</b>	2.5 $\mu$ l of PKC Lipid Activator (10X) (sonicate or vortex lipid for 1 minute prior to use)
<b>Component 4.</b>	7.5 $\mu$ l of 2X Buffer
- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 8.** At the same time as the PKC $\gamma$  kinase reaction, set up an ATP to ADP conversion curve at 50 $\mu$ M ATP/ADP range as described in the ADP-Glo™ Kinase Assay technical Manual #TM313.
- Step 9.** Initiate the PKC $\gamma$  reactions by the addition of 5 $\mu$ l of 250 $\mu$ M ATP Assay Solution thereby bringing the final volume up to 25 $\mu$ l. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25 $\mu$ l of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50 $\mu$ l of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLU, see ADP-Glo™ Applications Database at <http://www.promega.com/applications/cellularanalysis/cellsignaling.htm>

### Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol / (Reaction time in min)\*(Enzyme amount in mg)