

# PKC $\gamma$ Kinase

✓ 5  $\mu$ g

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com

**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com

**Web** ■ www.cellsignal.com

New 06/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full-length human PKC $\gamma$  kinase, supplied as a GST fusion protein.

**Background: Background:** Activation of PKC is one of the earliest events in a cascade leading to a variety of cellular responses such as secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms have been classified into three groups: classical PKCs, which are Ca<sup>2+</sup> dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG) and phorbol esters (TPA or PMA) through their cysteine-rich C1 domains, novel PKCs, which are Ca<sup>2+</sup> independent but are still regulated by PS, DAG and TPA and atypical PKCs, which are Ca<sup>2+</sup> independent and do not require PS, DAG or TPA for their activation (3-7). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to the substrate binding site in the catalytic domain, preventing its activation in the absence of cofactors or activators.

Other members have been recently added to the PKC superfamily based on homology within the catalytic domain. PKC, or PKD, is regulated by DAG and TPA through its C1 domain. However, PKD is distinguished by a PH domain, as well as by its unique substrate recognition and Golgi localization. The PKC-related kinases, or PRKs, lack a C1 domain and thus do not respond to DAG or phorbol esters. Instead, they can be activated by phosphatidylinositol lipids and their kinase activity is directly regulated by small GTPases of the Rho family through Rho binding to the homology region 1 (HR1).

The activity of PKC is under the control of three distinct phosphorylation events. Specifically, Thr500 in the activation loop, the Thr641 autophosphorylation site and the Ser660 hydrophobic site at the carboxy terminus of PKC $\beta$  II are phosphorylated *in vivo* (2). For the atypical

PKC isoforms, there is no phosphorylation in the hydrophobic region, which has a glutamic acid residue in place of the serine or threonine residue found in other PKC isoforms. The enzyme PDK1, or perhaps a close relative, is responsible for PKC activation.

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human PKC $\gamma$  (Met1-Met697) (GenBank Accession No. NM\_002739) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the PKC $\gamma$  fusion protein is 105 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PKC $\gamma$  kinase activity was determined using a radiometric assay [Fig.2].

#### Background References:

- (1) Nishizuka, Y. (1984) *Nature* 308, 693-698.
- (2) Keranen, L.M. et al. (1995) *Curr. Biol.* 5, 1394-1403.
- (3) Newton, A.C. (1995) *J. Biol. Chem.* 270, 28495-28498.
- (4) Mellor, H. and Parker, P.J. (1998) *Biochem J.* 332 (Pt 2), 281-292.
- (5) Ron, D. and Kazanietz, M.G. (1999) *FASEB J.* 13, 1658-1676.
- (6) Way, K.J. et al. (2000) *Trends Pharmacol. Sci.* 21, 181-187.
- (7) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep.* 1, 399-403.
- (8) Le Good, J.A. et al. (1998) *Science* 281, 2042-2045.
- (9) Sonnenburg, E.D. et al. (2001) *J. Biol. Chem.* 276, 45289-45297.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

#### Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

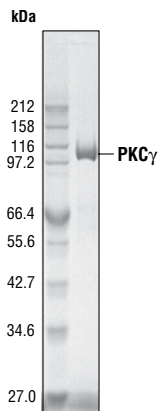


Figure 1. The purity of the PKC $\gamma$  fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

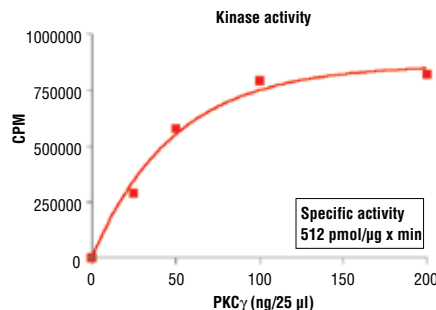


Figure 2. PKC $\gamma$  kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50  $\mu$ M ATP. Substrate: PKC substrate peptide 300 ng/ $\mu$ L, and variable amounts of recombinant PKC $\gamma$ .

## Protocol for PKC $\gamma$ Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

#### 1. Kinase Buffer (10X)

50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM MgCl<sub>2</sub>  
0.5 mM DTT

#### 2. ATP (10 mM) #9804

#### 3. <sup>32</sup>P- $\gamma$ ATP

#### 4. Lipid activator

20 mM MOPS  
0.5 mg/ml phosphatidylserine  
0.05 mg/ml diacylglycerol  
1 mM CaCl<sub>2</sub>

#### 5. PKC substrate peptide (ERM $\gamma$ PRKRQGSVRRRV, 1 $\mu$ g/ $\mu$ l)

### B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
2. Dilute [<sup>32</sup>P] ATP to 0.16  $\mu$ Ci/ $\mu$ l [<sup>32</sup>P] ATP with 250  $\mu$ M ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute PKC $\gamma$  protein to 20 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10  $\mu$ l diluted PKC $\gamma$  kinase solution, 10  $\mu$ l PKC substrate peptide (1.0 ng/ $\mu$ l), and 2.5  $\mu$ l lipid activator, and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [<sup>32</sup>P] ATP solution.

### Final Assay Conditions

5 mM MOPS, pH 7.2  
2.5 mM  $\beta$ -glycerophosphate  
1 mM EGTA  
0.4 mM EDTA  
5 mM MgCl<sub>2</sub>  
0.1 mM CaCl<sub>2</sub>  
0.05 mM DTT  
0.05 mg/ml phosphatidylserine  
0.005 mg/ml diacylglycerol  
300 ng/ $\mu$ l PKC substrate peptide

6. After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com).