

# PKC $\beta$ II Kinase

✓ 5  $\mu$ g

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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 $\beta$  II kinase, supplied as a GST fusion protein.

**Background:** Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. Classical PKCs are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG) and phorbol esters (TPA, PMA) through their cysteine-rich C1 domains. Both novel and atypical PKCs are calcium-independent, but only novel PKCs are activated by PS, DAG and phorbol esters (3-5). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to substrate-binding site in the catalytic domain to prevent activation in the absence of cofactors or activators.

Control of PKC activity is regulated through three distinct phosphorylation events. Phosphorylation of Thr500 in the activation loop, the autophosphorylation site at Thr641 and at carboxy-terminal hydrophobic site Ser660 occurs *in vivo* (2). Atypical PKC isoforms lack hydrophobic region phosphorylation, which correlates with the presence of glutamic acid rather than the serine or threonine residues found in more typical PKC isoforms. Either the enzyme PDK1 or a close relative is responsible for PKC activation.

A recent addition to the PKC superfamily is PKC $\mu$  (PKD), which is regulated by DAG and TPA through its C1 domain. PKD is distinguished by the presence of a PH domain and by its unique substrate recognition and Golgi localization (6). PKC-related kinases (PRK) lack the C1 domain and do not respond to DAG or phorbol esters. Phosphatidylinositol lipids activate PRKs and small Rho-family GTPases bind to the homology region 1 (HR1) to regulate PRK kinase activity (7).

Both PKC $\beta$  I and PKC $\beta$  II are formed from a single gene locus (PKC $\beta$ ) by alternative splicing of the carboxy-terminal exons. PKC $\beta$ s are the major PKC isoforms expressed in a variety of tissues and function in various signaling pathways regulating proliferation, differentiation, metabolism and cell-type-specific functions (10). In colon cancer, PKC $\beta$  II appears to be overexpressed early in the carcinogenic process while PKC $\beta$  I expression decreases later in tumor development (11).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human PKC $\beta$  II (Met1-Ser673) (GenBank Accession No. X07109) 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 GST-PKC $\beta$  II fusion protein is 104 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain and Western blot [Fig.1]. PKC $\beta$  II kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure PKC $\beta$  II activity using HTScan® PKC $\beta$  II Kinase Assay Kit #7585 [Fig.3].

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

#### Companion Products:

HTScan® PKC $\beta$  II Kinase Assay Kit #7585

CREB (Ser133) Biotinylated Peptide #1331

Phospho-PKA Substrate (RRXS/T) (100G7E) Rabbit mAb #9624

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

#### Background References:

- (1) Nishizuka, Y. (1984) *Nature* 308, 693-698.
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- (4) Mellor, H. and Parker, P.J. (1998) *Biochem. J.* 332, 281-292.
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- (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.
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- (9) Sonnenburg, E.D. (2001) *J. Biol. Chem.* 276, 45289-45297.
- (10) Kawakami, T. et al. (2002) *J. Biochem. (Tokyo)* 132, 677-82.
- (11) Mackay, H.J. and Twelves, C.J. (2003) *Endocr. Relat. Cancer* 10, 389-96.

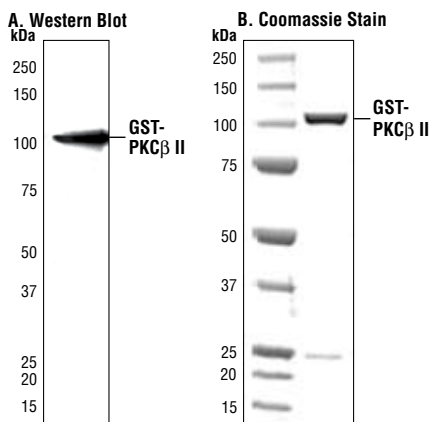


Figure 1. The purity of the GST-PKC $\beta$  II fusion protein was analyzed using SDS/PAGE followed by anti-GST Western blot (A) or Coomassie stain (B).

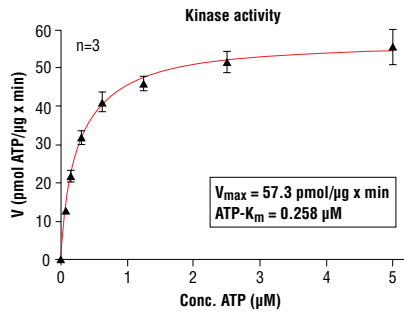


Figure 2. PKC $\beta$  II kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl<sub>2</sub>, 3 mM MnCl<sub>2</sub>, 3 µM Na-orthovanadate, 1.2 mM DTT, 1 µM ATP, 2.5 µg/50 µl PEG20,000, Substrate: Histone H1, 1 µg/50 µl, and Recombinant PKC $\beta$  II: 10 ng/50 µl.

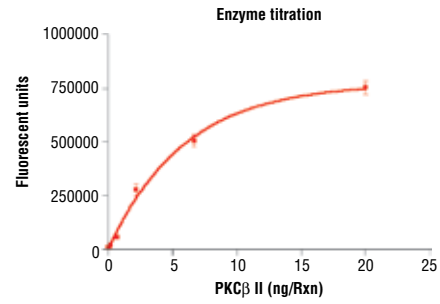


Figure 3. Dose dependence curve of PKC $\beta$  II kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of substrate peptide (#1331) by PKC $\beta$  II kinase. In a 50 µl reaction, increasing amounts of PKC $\beta$  II and 1.5 µM substrate peptide were used per reaction at room temperature for 15 minutes. (DELFLIA® is a registered trademark of PerkinElmer, Inc.)

## Protocol for PKC $\beta$ II Kinase Assay

**\*IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

### Kinase

**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. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. CREB (Ser133) Biotinylated Peptide #1331
8. DELFIA<sup>®</sup> Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA<sup>®</sup> Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA<sup>®</sup> Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

*DELFIA<sup>®</sup> is a registered trademark of PerkinElmer Life Sciences*

### B Suggested Protocol for 100 Assays

1. Add 100  $\mu$ l 10 mM ATP to 1.25 ml 6  $\mu$ M substrate peptide. Dilute the mixture with dH<sub>2</sub>O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400  $\mu$ M, [substrate] = 3  $\mu$ M).
2. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 50 mM  $\beta$ -glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH<sub>2</sub>O to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=0.8 ng/ $\mu$ l in 4X reaction cocktail).
6. Add 12.5  $\mu$ l of the 4X reaction cocktail to 12.5  $\mu$ l/well of prediluted compound of interest (usually around 10  $\mu$ M) and incubate for 5 minutes at room temperature.
7. Add 25  $\mu$ l of 2X ATP/substrate cocktail to 25  $\mu$ l/well preincubated reaction cocktail/compound.

### Final Assay Conditions for a 50 $\mu$ l Reaction

- 25 mM Tris-HCl (pH 7.5)
  - 10 mM MgCl<sub>2</sub>
  - 5 mM  $\beta$ -glycerophosphate
  - 0.1 mM Na<sub>3</sub>VO<sub>4</sub>
  - 2 mM DTT
  - 200  $\mu$ M ATP
  - 1.5  $\mu$ M peptide
  - 10 ng PKC $\beta$  II Kinase
8. Incubate reaction plate at room temperature for 15 minutes.
  9. Add 50  $\mu$ l/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
  10. Transfer 25  $\mu$ l of each reaction to a 96-well streptavidin-coated plate containing 75  $\mu$ l dH<sub>2</sub>O/well and incubate at room temperature for 60 minutes.
  11. \*Wash three times with 200  $\mu$ l/well PBS/T.
  12. Dilute primary antibody in PBS/T with 1% BSA. Add 100  $\mu$ l/well primary antibody.  
**Please note:** This protocol was validated using a CREB (Ser133) Biotinylated Peptide and Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
  13. Incubate at 37°C for 120 minutes.
  14. \*Wash three times with 200  $\mu$ l/well PBS/T.
  15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100  $\mu$ l/well diluted antibody.
  16. Incubate at room temperature for 30 minutes.
  17. \*Wash five times with 200  $\mu$ l/well PBS/T.
  18. Add 100  $\mu$ l/well DELFIA<sup>®</sup> Enhancement Solution.
  19. Incubate at room temperature for 5 minutes.
  20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.  
Email: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com)