Store at -80°C

#7608

PKCζ Kinase

☑5 µg

Cell Signaling TECHNOLOGY®

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new 01/06

Description: Purified recombinant full length human PKCζ kinase, supplied as a GST fusion protein.

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^{2+} 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^{2+} independent but are still regulated by PS, DAG and TPA; and atypical PKCs, which are Ca^{2+} 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 PKCβ 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.

PKCζ is one of the downstream effectors of phosphoinositide 3-kinase and it is required for cell mitogen-activated protein kinase cascade, transcriptional factor NFκB activation, ribosomal S6-protein kinase signaling, and cell polarity. In tumor cells exposed cytotoxic agents, PKCζ is thought to be involved in transducing cell survival signals that contribute to chemotherapeutic resistance (10,11).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human PKCζ (Met1-Val584) (GenBank Accession No. Z15108) 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-PKCζ fusion protein is 97 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain and Western blot [Fig.1]. PKCζ kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure PKCzeta activity using HTScan™ PKCζ Kinase Assay Kit #7607 [Fig.3].

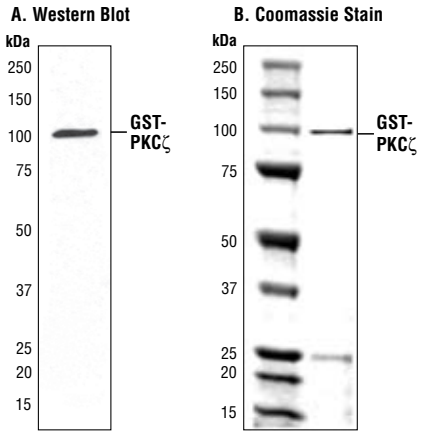

Figure 1. The purity of the GST-PKCζ fusion protein was analyzed using SDS/PAGE followed by anti-GST Western blot (A) or Coomassie stain (B).

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, 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) Good, J.A. et al. (1998) *Science* 281, 2042–2045.

(9) Sonnenburg, E.D. (2001) *J. Biol. Chem.* 276, 45289–45297.

(10) Filomenko, R. et al. (2002) *Cancer Res.* 62, 1815–21.

(11) Mackay, H.J. and Twelves, C.J. (2003) *Endocr. Relat. Cancer* 10, 389–96.

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™ PKCζ Kinase Assay Kit #7607

Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624

CREB (Ser133) Biotinylated Peptide #1331

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

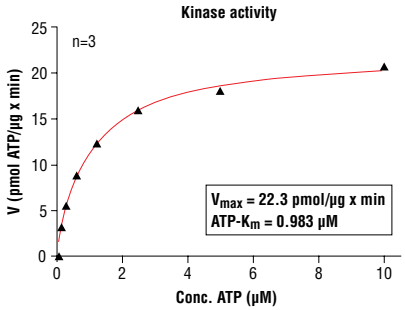

Figure 2. PKCζ kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 5 mM $MgCl_2$, 1.32 mM $CaCl_2$, 1 mM EDTA, 1.25 mM EGTA, 0.25 µg/50 µl phosphatidylserine, 50 ng/50 µl 1.2 Dioleyl-glycerol, 1.2 mM DTT, ATP: variable, 2.5 µg/50 µl PEG20.000, Substrate: PKC(19-31) (RFARKGSLRQKNV), 1.0 µg/50 µl and Recombinant PKCζ: 200 ng/50 µl.

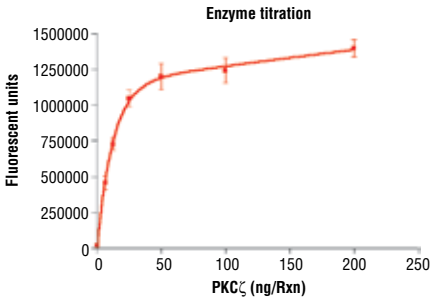

Figure 3. Dose dependence curve of PKCζ kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of substrate peptide (#1331) by PKCζ kinase. In a 50 µl reaction, increasing amounts of PKCζ and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for PKCζ 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® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
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 $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 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]=4.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng PKCζ Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/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 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® 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