#7815 Store at -80°C

HTScan® PKCε Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 09/05/07

Products Included	Products #	Kit Quantity
Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb	9624	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
CREB (Ser133) Biotinylated Peptide	1331	1.25 ml
PKCε Kinase (recombinant, human)	7492	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human PKCε kinase. It includes active PKCε kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine/threonine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: RRPS*YRK

Molecular Weights: Peptide substrate, Biotin-peptide: 2,326 Daltons. GST-PKCε Kinase domain: 110 kDa.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human PKCε (Met1-Pro737) (GenBank Accession No. NM_005400) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The substrate peptide was selected using our Serine/Threonine Kinase Substrate Screening Kit #7400. Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified PKCε kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. PKCε kinase specific activity was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose-dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify PKCε activity using the PKCε substrate peptide provided in this kit. PKCε sensitivity to the inhibitor staurosporine was measured using the PKCε substrate peptide provided in this kit [Fig.5].

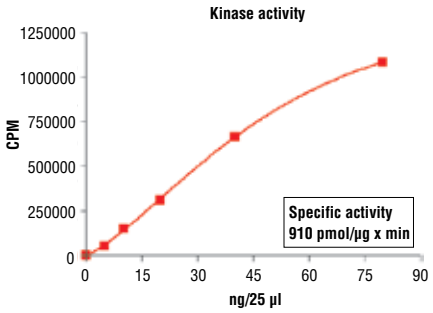

Figure 1. PKCε kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 40 ng/µl BSA, 50 µg/ml phosphatidylserine, 5 µg/ml diacylglycerol, 0.1 mM sodium orthovanadate, 0.1 mM dithioreitol, 0.1 mM $CaCl_2$, 50 µM ATP, Substrate: PKCε substrate peptide 300 ng/µL and recombinant PKCε: variable.

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Serine/Threonine Kinase Substrate Screening Kit #7400

PKCε Kinase #7492

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

CREB (Ser133) Biotinylated Peptide #1331

Staurosporine #9953

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

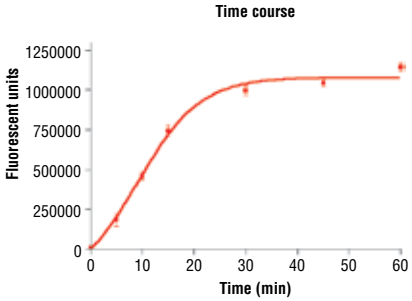

Figure 2. Time course of PKCε kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of PKCε substrate peptide (#1331) by PKCε kinase. In a 50 µl reaction, 25 ng PKCε and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

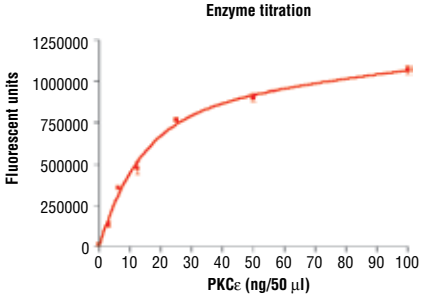

Figure 3. Dose dependence curve of PKCε kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of substrate peptide (#1331) by PKCε kinase. In a 50 µl reaction, increasing amounts of PKCε and 1.5 µM substrate peptide were used per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

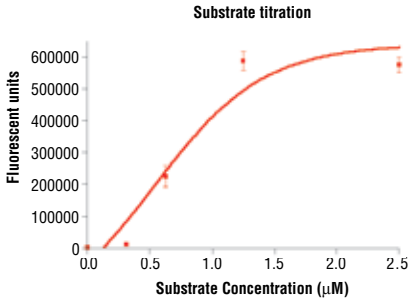

Figure 4. Peptide concentration dependence of PKCε kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of substrate peptide (#1331) by PKCε kinase. In a 50 µl reaction, 25 ng of PKCε and increasing concentrations of substrate peptide were used per reaction at room temperature for 15 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

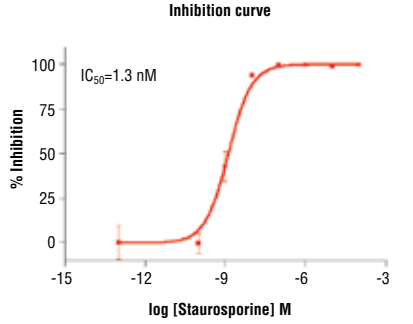

Figure 5. Staurosporine inhibition of PKCε kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of PKCε substrate peptide (#1331) by PKCε kinase. In a 50 µl reaction, 25 ng PKCε, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 15 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

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.

PKCε regulates various physiological functions and is involved in the activation of nervous, endocrine, exocrine, inflammatory and immune systems. Disruption of PKCε regulation has been related to diseases including cardiac ischemia, Alzheimer's, malignant tumors and diabetes (10).

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) B*iochem. 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) Akita, Y. (2002) *J. Biochem.* (Tokyo) 132, 847–852.

Protocol for HTScan® PKCε Kinase Assay Kit

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

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 [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. Transfer 1.2 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 4 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
25 ng PKCε 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, Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

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

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