Store at -80°C

#7694

PKD3/PKCν Kinase

☑5 µg

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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 PKD3/PKCν (Met1-Pro890) kinase, supplied as a GST fusion protein.

Background: PKCν, also known as PKD3, is a member of the protein kinase C (PKC) family of serine/threonine kinases that play critical roles in the regulation of cellular differentiation and proliferation. PKCν is composed of 890 amino acid residues and has 77.3% similarity to human PKCμ (PKCμ) and 77. 4% similarity to mouse PKD (the mouse homolog of PKCμ) (1). The PKCν mRNA is ubiquitously expressed in various tissues. PKCν has two putative diacylglycerol binding C1 domains, suggesting that it may participate in a novel diacylglycerol-mediated signaling pathway (2). PKCν is translocated to the plasma membrane and activated by the diacylglycerol mimic phorbol 12-myristate 13-acetate. PKCν is an important physiologic target of the B-cell receptor (BCR) and exhibits robust activation after BCR engagement (2). GPCR agonists induce a rapid activation of PKCν by a protein kinase C (PKC)-dependent pathway that leads to the phosphorylation of the activation loop of PKCν. PKCν is present both in the nucleus and cytoplasm and this distribution of PKCν results from its continuous shuttling between both compartments by a mechanism that requires a nuclear import receptor and a competent CRM1-nuclear export pathway (3). Cell stimulation with the GPCR agonist neurotensin induces a rapid and reversible plasma membrane translocation of PKCν that is PKC-dependent.

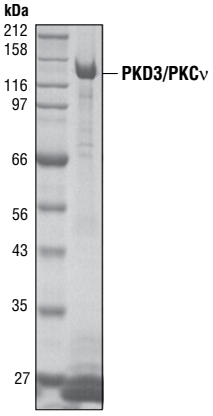

Figure 1. The purity of the GST-PKD3/PKCν fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human PKD3/PKCν (Met1-Pro890) (GenBank Accession No. NM_005813) 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-PKD3/PKCν fusion protein is 126 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PKD3/PKCν kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Hayashi, A. et al. (1999) *Biochim. Biophys. Acta.* 1450, 99–106.

(2) Matthews, S.A. et al. (2003) *J. Biol. Chem.* 278, 9086–91.

(3) Rey, O. et al. (2003) *J. Biol. Chem.* 278, 23773–85.

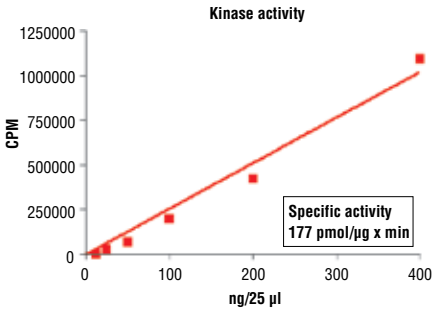

Figure 2. PKD3/PKCν kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 40 ng/µL BSA, 50 µM ATP, Substrate: CREBtide 400 ng/µL and recombinant PKD3/PKCν: variable.

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.

Protocol for PKD3/PKCν 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)**
   40 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   40 mM $MgC1_2$
   0.5 mM DTT
   400 ng/µl BSA
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. CREBtide (KRREILSRRPSYR) (1 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute PKD3/PKCν kinase protein (100 ng/µl concentration) to 50 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted PKD3/PKCν kinase solution, 10 µl CREBtide (1 µg/µl) and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay 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
400 ng/µL CREBtide

6. After 15 minutes terminate reaction by spotting 20 µl 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.

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