Store at -80°C

#7692

PKD2 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 PKD2 (Met1-Leu878) kinase, supplied as a GST fusion protein.

Background: PKD2 is a novel phorbol ester- and growth factor-stimulated serine/threonine kinase that contains two cysteine-rich motifs at the N terminus, a pleckstrin homology domain and a catalytic domain (1). It exhibits the strongest homology to the serine/threonine protein kinases PKD/PKCμ and PKCν, particularly in the duplex zinc finger-like cysteine-rich motif, in the pleckstrin homology domain and in the protein kinase domain. The PKD family of enzymes have been implicated in very diverse cellular functions, including Golgi organization and plasma membrane directed transport, metastasis, immune responses, apoptosis and cell proliferation (2). PKD2 can be activated by phorbol esters both *in vivo* and *in vitro* but also by gastrin via the cholecystokinin/CCK(B) receptor in human gastric cancer cells stably transfected with the CCK(B)/gastrin receptor (AGS-B cells) (3). Gastrin-stimulated PKD2 activation involves a heterotrimeric G α(q) protein as well as the activation of phospholipase C. Furthermore, PKD2 can be activated by classical and novel members of the protein kinase C (PKC) family such as PKCα, PKCε and PKCη implicating PKD2 to be a downstream target of specific PKCs upon the stimulation of AGS-B cells with gastrin. PKD2 is predominantly cytoplasmic and stimulation of cells with the G protein-coupled receptor agonist neurotensin induces a rapid and reversible plasma membrane translocation of PKD2 by a mechanism that requires PKC activity (4). In contrast to the other PKD isoenzymes, PKD2 activation does not induce its redistribution from the cytoplasm to the nucleus.

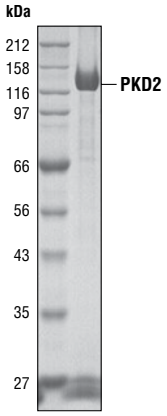

Figure 1. The purity of the GST-PKD2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human PKD2 (Met1-Leu878) (GenBank Accession No. NM_016457) 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-PKD2 fusion protein is 124 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PKD2 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Sturany, S. et al. (2001) *J. Biol. Chem.* 276, 3310–8.

(2) Rykx, A. et al. (2003) *FEBS Lett.* 546, 81–6.

(3) Sturany, S. et al. (2002) *J. Biol. Chem.* 277, 29431–6.

(4) Rey, O. et al. (2003) *Biochem. Biophys. Res. Commun.* 302, 817–24.

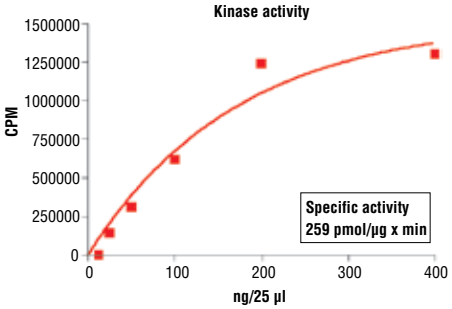

Figure 2. PKD2 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: CREBtide 400 ng/µL and recombinant PKD2: variable.

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.

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Protocol for PKD2 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
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 PKD2 protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted PDK2 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
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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