PKD2 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP76

Lot No. B208-1
5 µg 0.1 µg/µl

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/PKCmu and PKCnu. 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).

Product Description:

Recombinant full-length human PKD2 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_016457.

Gene Aliases:

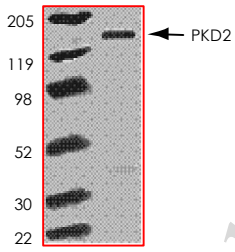
HSPC187; DKFZp586E0820; PRKD2

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >95% by densitometry. Approx. MW 130kDa.



Storage:

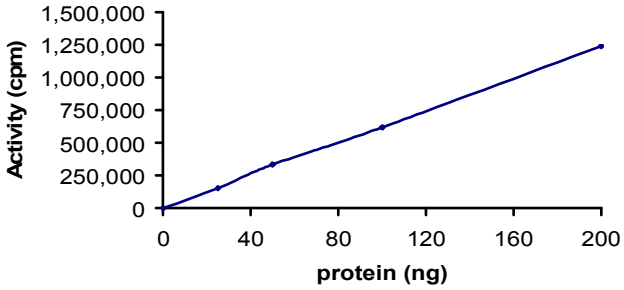
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at –70 °C, 1 year from date of shipment.

Specific Activity:

The specific activity was determined to be 259 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Sturany, S. et al: Molecular cloning and characterization of the human protein kinase D2. A novel member of the protein kinase D family of serine threonine kinases. J Biol Chem. 2001 Feb 2;276(5):3310-8.

2.Rykx, A. et al: Protein kinase D: a family affair. FEBS Lett. 2003 Jul 3;546(1):81-6.

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