PDK1 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP14

Lot No. A319-1
5 µg 0.1 µg/µl

Background:

PDK1 (3-phosphoinositide-dependent protein kinase) is activated by the presence of PtdIns (3, 4, 5) P3 or PtdIns (3, 4) P2 (1). PDK1 then activates protein kinase B (PKB) which, in turn, inactivates glycogen synthase kinase-3 (GSK3). The phosphorylation of other proteins by PKB and GSK3 is likely to mediate many of the intracellular actions of insulin. Thus, PDK1 plays a key role in mediating many of the actions of the second messenger(s) PtdIns (3, 4, 5) P3 and/or PtdIns (3, 4) P2. The human PDK1 is a 556-residue monomeric enzyme comprising of a catalytic domain that is most similar to the PKA, PKB and PKC subfamily of protein kinases.

Product Description:

Recombinant full-length human PDK1 was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_002613.

Gene Aliases:

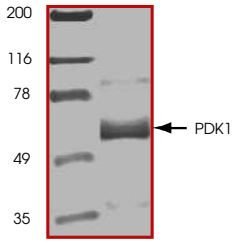
PRO0461; PDPK1

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 >80% by densitometry. Approx. MW 59kDa.



Storage:

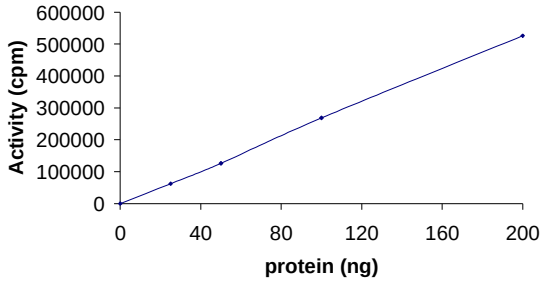
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 123 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:

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

5 X Kinase Assay Buffer:

25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Cohen, P. et al: PDK1, one of the missing links in insulin signal transduction? FEBS Letter. 1997 Jun 23;410(1):3-10. Review.

2.Alessi, DR. et al: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha. Curr Biol. 1997 Apr 1;7(4):261-9.

CycLex Co., Ltd
1063-103 Tera-Sawaoka, Ina, Nagano, Japan 396-0002
Fax: 81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp