

## Product Specification

### **PDK1, active**

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7706-5  
 Lot #: \_\_\_\_\_  
 Aliquot size: 5 µg protein in 50 µl  
 Specific activity: 159 nmol/min/mg

### **Quality Control Analysis**

#### Activity assessment

PDK1 protein (100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl<sub>2</sub>, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the PDKtide substrate peptide (KTFCGTPEYLAPEVRREPRILSEEEQEMFR DFDYIADWC) in the following assay condition:

- 10 µl diluted PDK1 protein
- 5 µl PDKtide substrate peptide (1 mg/ml stock)
- 5 µl water
- 5 µl [<sup>32</sup>P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30<sup>0</sup>C and the reaction started by the addition of [<sup>32</sup>P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

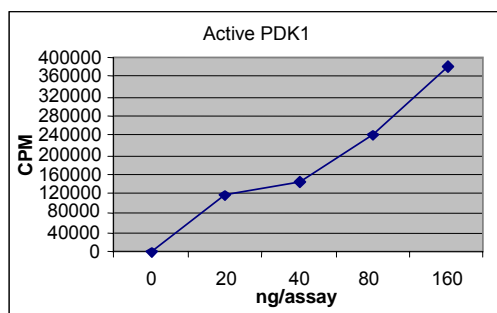


Fig. 1 PDK1 activity assay

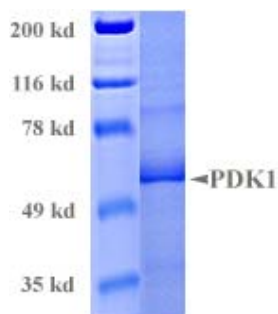


Fig. 2 PDK1 protein gel

#### Purity assessment

1 µg of PDK1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the PDK1 protein, and the band was at ~59 kDa (Fig. 2).

## **Product Description**

Recombinant full length human PDK1 containing N-terminal His tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM\_002613.

This material is sold for research purposes only.

## Specific Activity

159 nmol phosphate incorporated into PDKtide substrate peptide per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μM ATP (0.83 μCi/assay).

## Formulation

Recombinant proteins in storage buffer (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).

## Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

## Scientific Background

The initial steps in insulin signal transduction occur at the plasma membrane and lead to the activation of phosphatidylinositol (PtdIns) 3-kinase and the formation of PtdIns(3,4,5)P<sub>3</sub> in the inner leaflet of the plasma membrane which is then converted to PtdIns(3,4)P<sub>2</sub> by a specific phosphatase. PDK1 or 3-phosphoinositide-dependent protein kinase (PDK1), is activated by the presence of PtdIns(3,4,5)P<sub>3</sub> or PtdIns(3,4)P<sub>2</sub> (1). PDK1 then activates protein kinase B (PKB) (2) 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)P<sub>3</sub> and/or PtdIns(3,4)P<sub>2</sub>. 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 and a carboxy-terminal pleckstrin homology (PH) domain. The PDK1 gene is located on human chromosome 16p13.3 and is expressed ubiquitously in human tissues. Human PDK1 is homologous to the *Drosophila* protein kinase DSTPK61 (3), which has been implicated in the regulation of sex differentiation, oogenesis and spermatogenesis. Expressed PDK1 and DSTPK61 phosphorylated Thr308 of PKB alpha only in the presence of PtdIns(3,4,5)P<sub>3</sub> or PtdIns(3,4)P<sub>2</sub>. Overexpression of PDK1 in 293 cells activated PKB alpha and potentiated the IGF1-induced phosphorylation of PKB alpha at Thr308.

## References

1. Cohen P, Alessi DR, Cross DA. PDK1, one of the missing links in insulin signal transduction? FEBS Letter. 1997 Jun 23;410(1):3-10. Review.
2. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. 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.
3. Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, MacDougall CN, Harbison D, Ashworth A, Bownes M. 3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase. *Curr Biol*. 1997 Oct 1;7(10):776-89.