Recombinant Human Active PDK-1

Catalog Number: 864-KS-010
Lot Number: 1767581

SPECIFICATIONS AND USE

Source	Recombinant human PDK-1 was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His tag. The gene accession number is NM_002613.
Molecular Mass	The approximate molecular weight is 67 kDa.
Purity	The purity was determined to be > 70% by densitometry.
Formulation	Supplied in 50 mM Sodium Phosphate, pH 7.0, 300 mM NaCl, 150 mM Imidazole, 0.1 mM PMSF, 0.25 mM DTT, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of PDK-1 was determined to be 31 nmol/min/mg using a peptide substrate (see Activity Assay Protocol).
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

PDK-1 (3-phosphoinositide-dependent protein kinase) is activated by the presence of PtdIns (3,4,5) P3 or PtdIns (3,4) P2 (1). PDK-1 then activates protein kinase B (PKB) that, in turn, inactivates glycogen synthase kinase-3 (GSK-3). The phosphorylation of other proteins by PKB and GSK-3 is likely to mediate many of the intracellular actions of insulin. Thus, PDK-1 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. Human PDK-1 is a 556 amino acid residue monomeric enzyme comprised of a catalytic domain that is most similar to the PKA, PKB, and PKC subfamily of protein kinases.

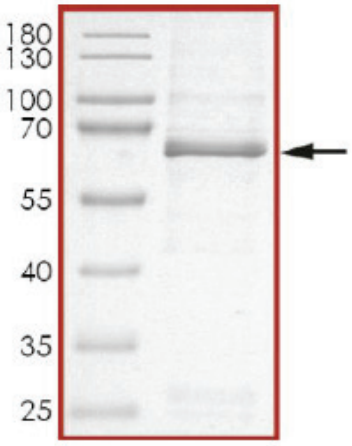

Figure 1: The approximate molecular weight is 67 kDa and the purity is > 70%.

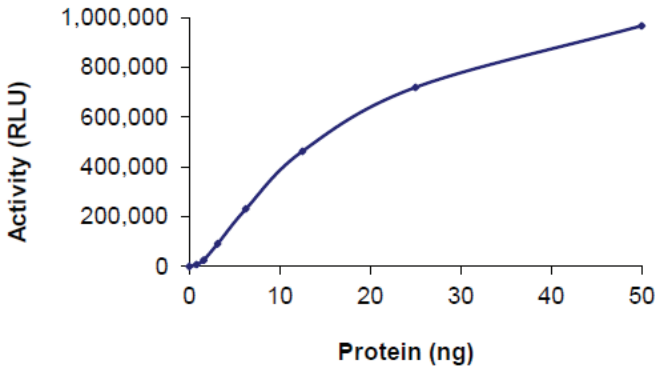

Figure 2: The specific activity of PDK1 was determined to be 31 nmol/min/mg as per Activity Assay Protocol, and was equivalent to 138 nmol/min/mg as per Radiometric Assay.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active PDK-1 (0.1 µg/µL) diluted with Kinase Dilution Buffer IX and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold distilled water. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - PDKtide synthetic peptide substrate (KTFCGTPEYLAPEV-RREPRILSEEEQEMFRDFDYIADWC) diluted in 40 mM Tris-HCl, pH 7.5 solution to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

The PDK-1 assay is performed using the ADP-Glo™ Kinase Assay Kit which quantifies the amount of ADP produced by the PDK-1 reaction. The ADP-Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active PDk-1, Kinase Assay Buffer lll (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1X), in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer lll (5x)	79 µL
Substrate at 1 mg/mL	80 µL

3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active PDK-1.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ATP-ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)]