

Product: PDK1, Active

Catalog #: 02-2079

Amount: 5 µg

Product Description

Recombinant full-length house PDK1 was expressed by baculovirus in Sf9 insect cells using a 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.

Storage and Stability

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.

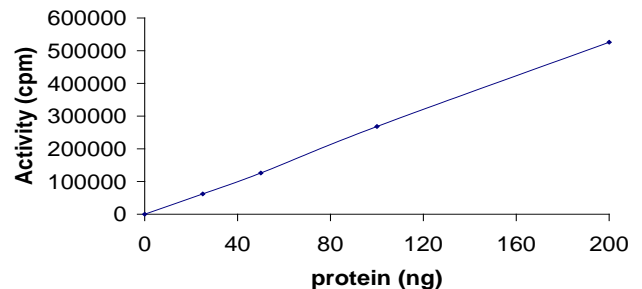
Scientific Background

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.

References

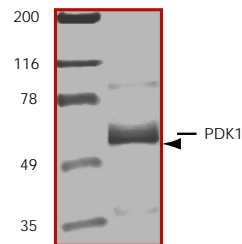
1. Cohen, P. et al: PDK1, one of the missing links in insulin signal transduction? FEBS Letter. 1997 Jun 23;410(1):3-10. Review.
2. Alessi, DR. et al: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha. Curr Biol. 1997 Apr 1;7(4):261-9.

Specific Activity



The specific activity of PDK1 was determined to be **123 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>80%** by densitometry. Approx. MW **59kDa**.

PDK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	02-2079
Quantity	5µg
Specific Activity	123 nmol/min/mg
Specific Lot Number	A319-1
Purity	>80%
Format	5µg in 50µl
Concentration	0.1µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2079)

Active PDK1 (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PDK1 for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

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

[γ]-ATP Assay Cocktail

Prepare 250µM [γ]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [32P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at –.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at –.

Substrate

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

Assay Protocol

- Step 1. Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PDK1, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1. 10µl of diluted Active PDK1 (Catalog #02-2079)
 - Component 2. 10µl of 1mg/ml stock solution of substrate
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5. Initiate the reaction by the addition of 5µl [32P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P³²]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [32P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of 32P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.