

Certificate of Analysis

PDK1 (52-end), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-452, 14-452-K, 14-452M

Parent Lot # D9AN019U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal, 6His-tagged, recombinant human PDK1, amino acids 52-end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 100% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

Specific Activity (Parent lot# D9AN019U): 188U/mg, where one unit of PDK1, active activity is defined as 1nmol phosphate incorporated into 100µM PDKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.69mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 5% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

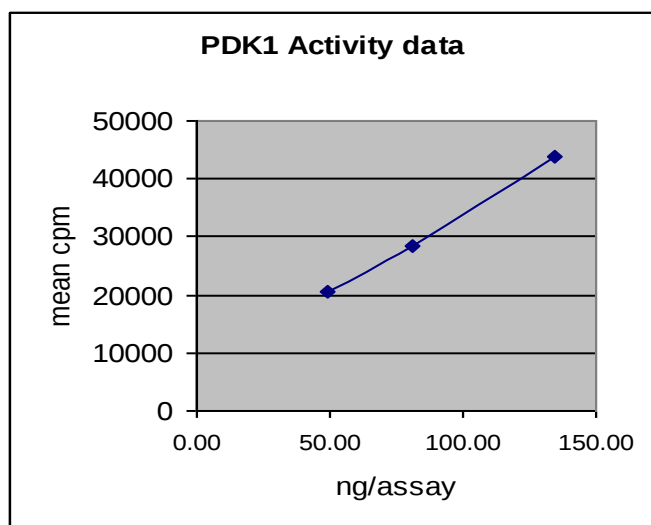
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C..

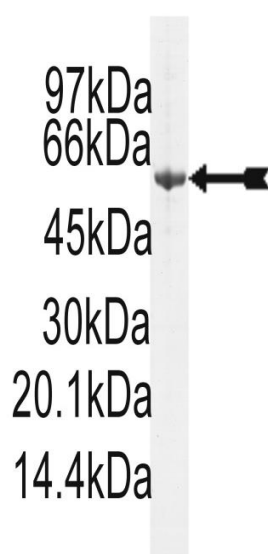
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 49–126ng of this lot of enzyme phosphorylated 100µM PDKtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as PDK1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PDK1, active.

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 0.5% 2-mercaptoethanol.
2. **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC):** Use at a final assay concentration of 100μM. Prepare a 1mM stock. Add 2.5μl of stock per assay point.
3. **PDK1.active:** Dilute with 50mM Tris/HCl pH7.5, 0.05% 2-mercaptoethanol, 1mg/ml BSA. Use 49–126ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5μl of reaction buffer per assay to wells.
2. Add 2.5μl of **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC)**.
3. Add **2.5μl (49–126ng) PDK1, active**.
4. Make up to 15μl with dH₂O.
5. Add 10μl of diluted [γ-³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5μl 3% phosphoric acid.
8. Transfer a 10μl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1μl of 30% phosphoric acid.

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PDK1 Sequence Information

Protein	Human PDK1
Tags	N-terminal 6His
Native sequence	D8 of the recombinant protein is equivalent to D52 of Human PDK1
Accession number	GenBank AF017995

Recombinant PDK1 amino acid sequence:

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1  MHHHHHDGT  AAEPRPGAGS  LQHAQPPPQP  RKKRPEDFKF  GKILGEGSFS  TVVLARELAT
61  SREYAIKILE  KRHIKENKV  PYVTRERDVM  SRLDHPFFVK  LYFTFQDDEK  LYFGLSYAKN
121 GELLKYIRKI  GSFDETCTRF  YTAEIVSALE  YLHGKGIHR  DLKPENILLN  EDMHIQITDF
181 GTAKVLSPES  KQARANSFVG  TAQYVSPPELL  TEKSACKSSD  LWALGCIYQ  LVAGLPPFRA
241 GNEYLIFQKI  IKLEYDFPEK  FFPKARDLVE  KLLVLDATKR  LGCEEMEGYG  PLKAHPFFES
301 VTWENLHQQT  PPKLTAYLPA  MSEDDEDCYG  NYDNLLSQFG  CMQVSSSSSS  HSLSASDTGL
361 PQRSNIEQ  YIHDLSNSF  ELDLQFSEDE  KRLLEKQAG  GNPWHQFVEN  NLILKMGPVD
421 KRKGLFARRR  QLLLTGPHL  YYVDPVNVKL  KGEIPWSQEL  RPEAKNFKTF  FVHTPNRTYY
481 LMDPSGNAHK  WCRKIQEVWR  QRYQSHPDAA  VQ

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Recombinant PDK1 nucleotide sequence:

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1  atgcatcacc  atcaccatca  tgacggcact  gcagccgagc  ctcggcccgg  cgccggctcc
61  ctgcagcatg  cccagcctcc  gccgcagcct  cggaagaagc  ggcctgagga  cttcaagttt
121 gggaaaatcc  ttggggaagg  ctctttttcc  acggttgctc  tggctcgaga  actggcaacc
181 tccagagaat  atgcgattaa  aattctggag  aagcgacata  tcataaaaga  gaacaaggct
241 ccctatgtaa  ccagagagcg  ggatgtcatg  tcgcgcctgg  atcacccctt  ctttgtttaag
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1441 ctgatggacc  ccagcgggaa  cgacacaaag  tgggtgcagga  agatccagga  ggtttggagg
1501 cagcgatacc  agagccacc  ggacgccgct  gtgcagtga

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