

## 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 Ni<sup>2+</sup>/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 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.

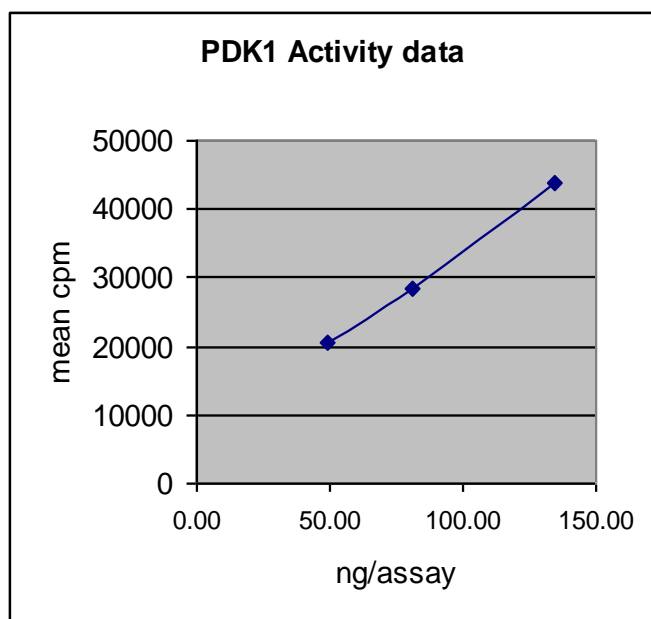
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

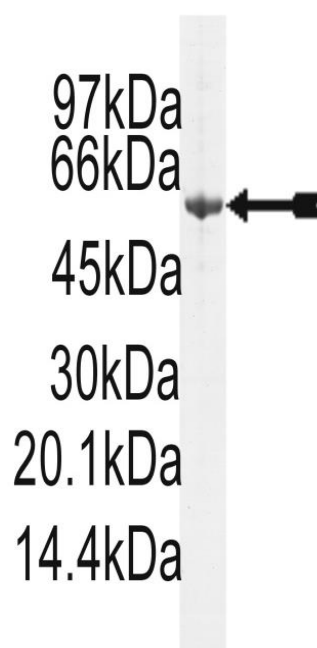
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### 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 active PDK1.

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### 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 100 $\mu$ M. Prepare a 1mM stock. Add 2.5 $\mu$ l 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. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 2.5 $\mu$ l of reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC)**.
3. Add **2.5 $\mu$ l (49–126ng) PDK1, active**.
4. Make up to 15 $\mu$ l with dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l 3% phosphoric acid.
8. Transfer a 10 $\mu$ l 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 1 $\mu$ l of 30% phosphoric acid.

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### PDK1 Sequence Information

<b><u>Protein</u></b>	Human PDK1
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	D8 of the recombinant protein is equivalent to D52 of Human PDK1
<b><u>Accession number</u></b>	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 GSFDECTRF YTAEIVSALE YLHGKGIHR DLKPENILLN EDMHIQITDF
181 GTAKVLSPEs KQARANSFVG TAQYVSPELL TEKSACKSSD LWALGCIIYQ LVAGLPPFRA
241 GNEYLIFQKI IKLEYDFPEK FFPKARDLVE KLLVLDATKR LGCEEMEGYG PLKAHPFFES
301 VTWENLHQQT PPKLTAYLPA MSEDDEDCYG NYDNLLSQFG CMQVSSSSSS HSLSASDTGL
361 PQRSGSNIEQ YIHDLSNSF ELDLQFSEDE KRLLEKQAG GNPWHQFVEN NLILKMGPDV
421 KRKGLFARRR QLLLTEGPHL 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 ctcttttcc acggttgtcc tggctcgaga actggcaacc
181 tccagagaat atgcgattaa aattctggag aagcgacata tcataaaaga gaacaaggtc
241 ccctatgtaa ccagagagcg ggatgtcatg tgcgcctgg atcacccctt ctttgtttaag
301 ctttacttca catttcagga cgacgagaag ctgtatttcg gccttagtta tgccaaaaat
361 ggagaactac ttaaatatat tcgcaaaatc ggttcattcg atgagacctg tacccgattt
421 tacacggctg agatcgtgtc tgctttagag tacttgcacg gcaagggcat cttcacagg
481 gaccttaaac cggaaaacat tttgttaaat gaagatatgc acatccagat cacagatttt
541 ggaacagcaa aagtcttatc cccagagagc aaacaagcca gggccaactc attcgtggga
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661 ctttgggctc ttggatgcat aatataccag cttgtggcag gactcccacc attccgagct
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1141 gaactggact tacagtttc cgaagatgag aagaggtgtg tgttggagaa gcaggctggc
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1381 cgaccagagg ccaagaattt taaaactttc tttgtccaca cgcctaacag gacgtattat
1441 ctgatggacc ccagcgggaa cgcacacaag tgggtgcagga agatccagga ggtttggagg
1501 cagcgatacc agagccacc ggacgccgct gtgcagtga
  
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