Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

PDK1

3-phosphoinositide dependent protein kinase-1

Recombinant Human Active Protein Kinase

HGNC Symbol: PDPK1

Synonyms: PKB-like

Product No.: 0367-0000-1

Lot: 002

Description: Human PDK1, N-terminal fragment, amino acids M_1-M_{460} (as in NCBI/Protein entry NP_002604.1), N-terminal HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PDK1 Lot 002, was confirmed as PDK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 56,192 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was not activated by special procedures

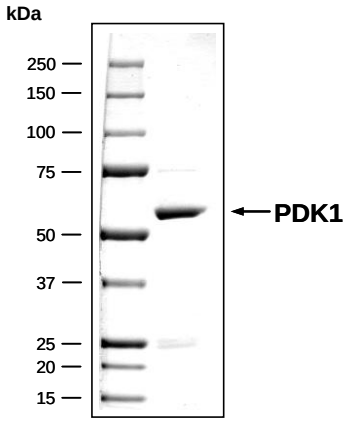
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 1.223 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

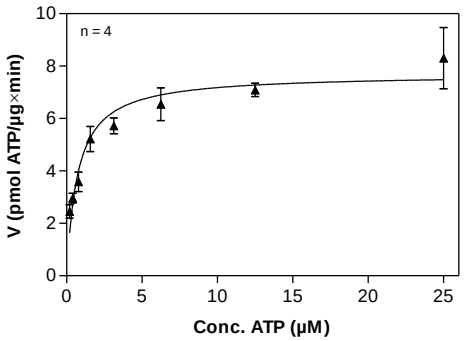
Biochemical Parameters:
Specific kinase activity (P_i transfer): 8 pmol/µg × min
ATP-K_M: 0.7 µM

PDK1 Lot 002:
Coomassie stain



2.0 µg PDK1

PDK1 Lot 002:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg / ml $PEG_{20.000}$
  ATP (variable)
  Substrate: tetra(LRRWSLG), 40 µg/ml
  PDK1: 0.8 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
PDK1 Lot 002 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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HIS-PDK1 Recombinant Fusion Protein Amino Acid Sequence

1	MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGICSR NSMARTTSQL YDAVPIQSSV	60
61	VLCSCPSPSM VRTQTESSTP PGIPGGSRQG PAMDGTAAEP RPGAGSLQHA QPPPQPRKKR	120
121	PEDFKFGKIL GEGSFSTVVL ARELATSREY AIKILEKRHI IKENKVPYVT RERDVMSRLD	180
181	HPFFVKLYFT FQDDEKLYFG LSYAKNGELL KYIRKIGSFD ETCTRFYTAE IVSALEYLHG	240
241	KGIIHRDLKP ENILLNEDMH IQITDFGTAK VLSPESKQAR ANSFVGTAQY VSPELLTEKS	300
301	ACKSSDLWAL GCIIYQLVAG LPPFRAGNEY LIFQKIIKLE YDFPEKFFPK ARDLVEKLLV	360
361	LDATKRLGCE EMEGYGPLKA HPFFESVTWE NLHQQTPPKL TAYLPAMSED DEDCYGNYDN	420
421	LLSQFGCMQV SSSSSSHSLS ASDTGLPQRS GSNIEQYIHD LDSNSFELDL QFSEDEKRLL	480
481	LEKQAGGNPW HQFVENNLIL KM	540

Red: HIS6-tag **Pink**: Thrombin cleavage site **blue**: PDK1 fragment

PDK1 wt[1] Amino Acid Sequence

1	MARTTSQLYD AVPIQSSVVL CSCPSPSMVR TQTESSTPPG IPGGSRQGPA MDGTAAEPRP	60
61	GAGSLQHAQP PPQPRKKRPE DFKFGKILGE GSFSTVVLAR ELATSREYAI KILEKRHIIK	120
121	ENKVPYVTRE RDVMSRLDHP FFVKLYFTFQ DDEKLYFGLS YAKNGELLKY IRKIGSFDET	180
181	CTRFYTAEIV SALEYLHGKG IIHRDLKPEN ILLNEDMHIQ ITDFGTAKVL SPESKQARAN	240
241	SFVGTAQYVS PELLTEKSAC KSSDLWALGC IIYQLVAGLP PFRAGNEYLI FQKIIKLEYD	300
301	FPEKFFPKAR DLVEKLLVLD ATKRLGCEEM EGYGPLKAHP FFESVTWENL HQQTPPKLTA	360
361	YLPAMSEDDE DCYGNYDNLL SQFGCMQVSS SSSSHSLSAS DTGLPQRSGS NIEQYIHDLD	420
421	SNSFELDLQF SEDEKRLLLE KQAGGNPWHQ FVENNLILKM GPVDKRKGLF ARRRQLLLTE	480
481	GPHLYYVDPV NKVLKGEIPW SQELRPEAKN FKTFFVHTPN RTYYLMDPSG NAHKWCRKIQ	540
541	EVWRQRYQSH PDAAVQ	600

blue: PDK1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002604.1

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