Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ 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

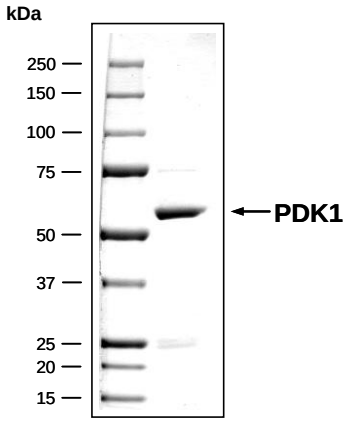
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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)

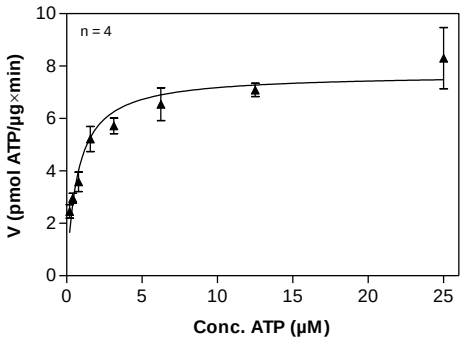
Biochemical Parameters:
Specific kinase activity (P_i transfer): 8 pmol/µg × min
ATP-K_M: 0.7 µM

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 Reaction Biology 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

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

PDK1 wt[1] Amino Acid Sequence

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  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
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blue: PDK1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002604.1

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