

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PDK1 [52 - 556]

<u>Enzyme description:-</u>	PDK1 [52 - 556]
<u>Clone number:-</u>	DU 954
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GST Sepharose
<u>Expression level:-</u>	3 mg/L
<u>Calculated molecular mass:-</u>	85, 047 daltons
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1% 2-mercaptoethanol, 0.1mM EGTA, 10 mM MgAc

Substrate:-

PDKtide (KTF CGTPEYL APEVRREPRILSEEEQEMFRDFDYIADWC)
Final concentration: 100µM

Specific activity range:- 60 - 120 U/mg

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Clone Data Sheet

PDK1 [52 – 556]

Protein PDK1 [52 - 556]

Clone number DU 954

Species Human

Accession no NM_002613

Tags N-terminal GST

Baculovirus expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKL TQSM A I I R Y I A D K H N M L G G C P K E R A E I S M L E
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVL F Q G P L G S P E F D G T A A E
PRPGAGSLQHAQPPPQPRKKRPEDFKFGKILGEGSFSTVVLARELATS
REYAIKILEKRHI I K E N K V P Y V T R E R D V M S R L D H P F F V K L Y F T F Q D D E
KLYFGLSYAKNGELLKYIRKIGSFDETCTRFYTAETVSALEYLHGKGI
IHRDLKPENILLNEDMHIQITDFGTAKVLSPEKQARANSFVGTAYV
SPELLTEKSACKSSDLWALGCIIYQLVAGLPPFRAGNEYLIFQKI I K L
EYDFPEKFFPKARDLVEKLLVLDATKRLGCEEMEGYGPLKAHPFFESV
TWENLHQQTTPPKLTAYLPAMSEDDDCYGNYNLLSQFGCMQVSSSS
SHSLSASDTGLPQRSGSNIEQYIHDLDSNSFELDLQFSEDEKRLLEK
QAGGNPWHQFVENNLILKMGVPDKRKGLFARRRQLLLTEGPHLYYVDP
VNVKVLKGEIPWSQELRPEAKNFKTFVHTPNRTYYLMDPSGNAHKWCR
KIQEVWRQRYQSHPDAAVQ

Native sequence Amino acids D52 – Q556 (end) of human PDK1.
Residue D235 of the fusion protein is equivalent to D52 of the native
enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Eco*R1 and *Sal*I of pFastBAC GST

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**Nucleotide
sequence of insert**

GAATTCGACGGCACTGCAGCCGAGCCTCGGCCCGGCGCCGGCTCCCTG
CAGCATGCCAGCCTCCGCCGAGCCTCGGAAGAAGCGGCCTGAGGAC
TTCAAGTTTGGGAAAATCCTTGGGGAAGGCTCTTTTTCCACGGTTGTC
CTGGCTCGAGAACTGGCAACCTCCAGAGAATATGCGATTAAAATCTG
GAGAAGCGACATATCATAAAAGAGAACAAGGTCCCCTATGTAACCAGA
GAGCGGGATGTCATGTGCGCGCTGGATCACCCCTTCTTTGTTAAGCTT
TACTTTCACATTTT CAGGACGACGAGAAGCTGTATTTTCGGCCTTAGTTAT
GCCAAAAATGGAGAACTACTTAAATATATTCGCAAAATCGGTTTCATTC
GATGAGACCTGTACCCGATTTTACACGGCTGAGATCGTGTCTGCTTTA
GAGTACTTGCACGGCAAGGGCATCATTACAGGGACCTTAAACCGGAA
AACATTTTGTAAATGAAGATATGCACATCCAGATCACAGATTTTGG
ACAGCAAAAGTCTTATCCCCAGAGAGCAAACAAGCCAGGGCCAACCTCA
TTCGTGGGAACAGCGCAGTACGTTTCTCCAGAGCTGCTCACGGAGAAG
TCCGCCTGTAAGAGTTCAGACCTTTGGGCTCTTGGATGCATAATATAC
CAGCTTGTGGCAGGACTCCACCATTCCGAGCTGGAAACGAGTATCTT
ATATTT CAGAAGATCATTAAGTTGGAATATGACTTTCAGAAAAATTC
TTCCCTAAGGCAAGAGACCTCGTGGAGAACTTTTGGTTTTAGATGCC
ACAAAGCGGTTAGGCTGTGAGGAAATGGAAGGATACGGACCTCTTAAA
GCACACCCGTTCTTCGAGTCCGTCACGTGGGAGAACCTGCACCAGCAG
ACGCCTCCGAAGCTCACCGCTTACCTGCCGGCTATGTCGGAAGACGAC
GAGGACTGCTATGGCAATTATGACAATCTCCTGAGCCAGTTTGGCTGC
ATGCAGGTGTCTTCGTCCTCCTCCTCACACTCCCTGTCAGCCTCCGAC
ACGGCCCTGCCCCAGAGGTCAGGCAGCAACATAGAGCAGTACATTCAC
GATCTGGACTCGAACTCCTTTGAACTGGACTTACAGTTTTCCGAAGAT
GAGAAGAGGTTGTTGTTGGAGAAGCAGGCTGGCGGAAACCCTTGGCAC
CAGTTTGTAGAAAATAATTTAATACTAAAGATGGGCCAGTGGATAAG
CGGAAGGGTTTTATTTGCAAGACGACGACAGCTGTTGCTCACAGAAGGA
CCACATTTATATTATGTGGATCCTGTCAACAAAGTTCTGAAAGGTGAA
ATTCCTTGGTCACAAGAACTTCGACCAGAGGCCAAGAATTTTAAAAC
TTCTTTGTCCACACGCCTAACAGGACGTATTATCTGATGGACCCACG
GGGAACGCACACAAGTGGTGCAGGAAGATCCAGGAGGTTTGGAGGCAG
CGATACCAGAGCCACCCGGACGCCGCTGTGCAGtgagtcgac