*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of active PDK1 [52 - 556]

**Enzyme description:-** PDK1 [52 - 556]

**Clone number:-** DU 831

**Source:-** Recombinant

**Expression system:-** Baculovirus expression vector system

**Tag:-** N-terminal His(6)

**Purification method**:- $Ni^{2+}$-NTA agarose

**Expression level:-** 3-5 mg/L

**Calculated molecular mass:-** 62, 724 daltons

**Purity:-** >80 %

**Activation protocol:-** 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 (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC)
Final concentration: 100 µM

**Specific activity range:-** 120 – 240 U/mg

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# Clone Data Sheet

## PDK1 [52 – 556]

| | |
|---|---|
| **Protein** | PDK1 [52 - 556] |
| **Clone number** | DU 831 |
| **Species** | Human |
| **Accession no** | NM_002613 |
| **Tags** | N-terminal His(6) and MYC (EQKLISEEDL) |
| **Baculovirus expressed protein** | MSYYHHHHHHDYDIPTTENLYFQGAMGSATMEQKLISEEDL**DGTAAEP RPGAGSLQHAQPPPQPRKKRPEDFKFGKILGEGSFSTVVLARELATSR EYAIKILEKRHIIKENKVPYVTRERDVMSRLDHPFFVKLYFTFQDDEK LYFGLSYAKNGELLKYIRKIGSFDETCTRFYTAEIVSALEYLHGKGII HRDLKPENILLNEDMHIQITDFGTAKVLSPESKQARANSFVGTAQYVS PELLTEKSACKSSDLWALGCIIYQLVAGLPPFRAGNEYLIFQKIIKLE YDFPEKFFPKARDLVEKLLVLDATKRLGCEEMEGYGPLKAHPFFESVT WENLHQQTPPKLTAYLPAMSEDDEDCYGNYDNLLSQFGCMQVSSSSSS HSLSASDTGLPQRSGSNIEQYIHDLDSNSFELDLQFSEDEKRLLLEKQ AGGNPWHQFVENNLILKMGPVDKRKGLFARRRQLLLTEGPHLYYVDPV NKVLKGEIPWSQELRPEAKNFKTFFVHTPNRTYYLMDPSGNAHKWCRK IQEVWRQRYQSHPDAAVQ** |
| **Native sequence** | Amino acids D52 – Q556 (end) of human PDK1.<br>Residue D42 of the fusion protein is equivalent to D52 of the native enzyme. The His(6) tag is located at residues 5 - 10 and the MYC tag is located at residues 32 - 41. |
| **Protease cleavage** | rTEV (ENLYFQG) residues 18 – 24 |
| **Cloning sites** | *Bam*HI and *Kpn*I sites of pFastBAC HTb |

**Complete nucleotide sequence**

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTTCAGGGCGCCATGGGATCTGCCACCATGGAG
CAGAAGCTGATCTCTGAAGAGGACTTGGACGGCACTGCAGCCGAGCCT
CGGCCCGGCGCCGGCTCCCTGCAGCATGCCCAGCCTCCGCCGCAGCCT
CGGAAGAAGCGGCCTGAGGACTTCAAGTTTGGGAAAATCCTTGGGGAA
GGCTCTTTTTCCACGGTTGTCCTGGCTCGAGAACTGGCAACCTCCAGA
GAATATGCGATTAAAATTCTGGAGAAGCGACATATCATAAAAGAGAAC
AAGGTCCCCTATGTAACCAGAGAGCGGGATGTCATGTCGCGCCTGGAT
CACCCCTTCTTTGTTAAGCTTTACTTCACATTTCAGGACGACGAGAAG
CTGTATTTCGGCCTTAGTTATGCCAAAAATGGAGAACTACTTAAATAT
ATTCGCAAAATCGGTTCATTCGATGAGACCTGTACCCGATTTTACACG
GCTGAGATCGTGTCTGCTTTAGAGTACTTGCACGGCAAGGGCATCATT
CACAGGGACCTTAAACCGGAAAACATTTTGTTAAATGAAGATATGCAC
ATCCAGATCACAGATTTTGGAACAGCAAAAGTCTTATCCCCAGAGAGC
AAACAAGCCAGGGCCAACTCATTCGTGGGAACAGCGCAGTACGTTTCT
CCAGAGCTGCTCACGGAGAAGTCCGCCTGTAAGAGTTCAGACCTTTGG
GCTCTTGGATGCATAATATACCAGCTTGTGGCAGGACTCCCACCATTC
CGAGCTGGAAACGAGTATCTTATATTTCAGAAGATCATTAAGTTGGAA
TATGACTTTCCAGAAAAATTCTTCCCTAAGGCAAGAGACCTCGTGGAG
AAACTTTTGGTTTTAGATGCCACAAAGCGGTTAGGCTGTGAGGAAATG
GAAGGATACGGACCTCTTAAAGCACACCCGTTCTTCGAGTCCGTCACG
TGGGAGAACCTGCACCAGCAGACGCCTCCGAAGCTCACCGCTTACCTG
CCGGCTATGTCGGAAGACGACGAGGACTGCTATGGCAATTATGACAAT
CTCCTGAGCCAGTTTGGCTGCATGCAGGTGTCTTCGTCCTCCTCCTCA
CACTCCCTGTCAGCCTCCGACACGGGCCTGCCCCAGAGGTCAGGCAGC
AACATAGAGCAGTACATTCACGATCTGGACTCGAACTCCTTTGAACTG
GACTTACAGTTTTCCGAAGATGAGAAGAGGTTGTTGTTGGAGAAGCAG
GCTGGCGGAAACCCTTGGCACCAGTTTGTAGAAAATAATTTAATACTA
AAGATGGGCCCAGTGGATAAGCGGAAGGGTTTATTTGCAAGACGACGA
CAGCTGTTGCTCACAGAAGGACCACATTTATATTATGTGGATCCTGTC
AACAAAGTTCTGAAAGGTGAAATTCCTTGGTCACAAGAACTTCGACCA
GAGGCCAAGAATTTTAAAACTTTCTTTGTCCACACGCCTAACAGGACG
TATTATCTGATGGACCCCAGCGGGAACGCACACAAGTGGTGCAGGAAG
ATCCAGGAGGTTTGGAGGCAGCGATACCAGAGCCACCCGGACGCCGCT
GTGCAGtga