

Standard Operating Procedure

Preparation of active PDK1

Enzyme description:-	Active PDK1 Δ 1-50
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system (BEVS)/Insect cells
<u>Tag:-</u>	His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose.
<u>Expression level:-</u>	3-5 mg/L
<u>Molecular mass:-</u>	67 kDa by SDS-PAGE
<u>Purity:-</u>	>80%
<u>Contaminants:-</u>	No major contaminating proteins as judged by Novex gel.
<u>Activation protocol:-</u>	None- constitutively active
<u>Enzyme storage buffer:-</u>	50 mM Tris/HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β -mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	Aliquot, snap freeze and store at -70°C.

University of Dundee

CLONE DATA SHEET –Human PDK1

Protein	Human PDK1 (His ₆ -myc-[Δ1 - 51] PDK1)
Accession no	AFO17995
Tags	His6 and Amino-terminal c-myc (EQKLISEEDL)
Baculovirus-expressed protein	MSYYHHHHHHHDYDIPTTENLYFQGAMGSATMEQKLISEEDLD GTAAEPRPGAGSLQHAQPPPQPRKKRPEDFKFGKILGEGSFST VVLARELATSREYAIIKLEKRHHIENKVPYVTRERDVMSRLDH PFFVKLYFTFQDDEKLYFGLSYAKNGELLKYIRKIGSFDETCTRF YTAEIVSALEYLHGKGIIHRDLKPENILLNEDMHIQITDFGTAK VLSPEKQARANSFVGTAQYVSPELLTEKSACKSSDLWALGCII YQLVAGLPPFRAGNEYLIQKIIKLEYDFPEKFFPKARDLVEKLL VLDATKRLGCEEMEGYGPLKAHPFFESVTWENLHQQTPPKLT AYLPAMSEDDDCYGNYNLLSQFGCMQVSSSSSSSHLSASDT GLPQRSGSNIEQYIHDLDNSFELDLQFSEDEKRLLEKQAGGN PWHQFVENNLILKMGPVDKRGKGLFARRRQLLLTEGPHLYYVD PVNKKVLKGEIPWSELRPEAKNFKTFFVHTPNRTYYLMDPSGNA HKWCRKIQEVWRQRYQSHPDAAVQ
native sequence	Aspartate 42 of the His ₆ -tagged protein is equivalent to Asp52 of full-length PDK1. The fusion protein contains an amino-terminal His6 tag (residues 5-10) and c-myc (EQKLISEEDL residues 32-41).
Protease cleavage site	ENLYFQ (rTEV protease) residues 18 – 23 of His ₆ -tagged protein

Cloning sites

A novel *Bgl*III site and c-myc epitope sequence were introduced immediately 5' to codon 52 by PCR. The resulting *Bgl*III/*Kpn*I fragment was sub-cloned into the *Bam*HI/*Kpn*I sites of pFastBAC HTb.

**ORF in
baculovirus**

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTTCAGGGCGCCATGGGATCTGCCACCATGGAG
CAGAAGCTGATCTCTGAAGAGGACTTGGACGGCACTGCAGCCGAGCCT
CGGCCCCGGCGCCGGCTCCCTGCAGCATGCCCAGCCTCCGCCGCAGCCT
CGGAAGAAGCGGCCTGAGGACTTCAAGTTTGGGAAAATCCTTGGGGAA
GGCTCTTTTTCACGGTTGTCTGGCTCGAGAACTGGCAACCTCCAGA
GAATATGCGATTAAAATTCTGGAGAAGCGACATATCATAAAAGAGAAC
AAGGTCCCCTATGTAACCAGAGAGCGGGATGTCATGTCGCGCCTGGAT
CACCCCTTCTTTGTAAAGCTTTACTTCACATTTTCAGGACGACGAGAAG
CTGTATTTTCGGCCTTAGTTATGCCAAAAATGGAGAACTACTTAAATAT
ATTTCGCAAAATCGGTTTCATTCGATGAGACCTGTACCCGATTTTACACG
GCTGAGATCGTGTCTGCTTTAGAGTACTTGCACGGCAAGGGCATCATT
CACAGGGACCTTAAACCGGAAAACATTTTGTAAATGAAGATATGCAC
ATCCAGATCACAGATTTTGGAAACAGCAAAAGTCTTATCCCCAGAGAGC
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CCAGAGCTGCTCACGGAGAAGTCCGCCTGTAAGAGTTCAGACCTTTGG
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CGAGCTGGAAACGAGTATCTTATATTTTCAGAAGATCATTAAGTTGGAA
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TGGGAGAACCTGCACCAGCAGACGCCTCCGAAGCTCACCGCTTACCTG
CCGGCTATGTGCGGAAGACGACGAGGACTGCTATGGCAATTATGACAAT
CTCCTGAGCCAGTTTGGCTGCATGCAGGTGTCTTCGTCTCCTCCTCA
CACTCCCTGTGAGCCTCCGACACGGGCCTGCCCCAGAGGTCAGGCAGC
AACATAGAGCAGTACATTCACGATCTGGACTCGAACTCCTTTGAACTG
GACTTACAGTTTTCCGAAGATGAGAAGAGGTTGTTGTTGGAGAAGCAG
GCTGGCGGAAACCCCTTGGCACCAGTTTGTAGAAAATAATTTAATACTA
AAGATGGGCCCAGTGGATAAGCGGAAGGGTTTATTTGCAAGACGACGA
CAGCTGTTGCTCACAGAAGGACCACATTTATATTATGTGGATCCTGTC
AACAAAGTTCTGAAAGGTGAAATTCCTTGGTCCACAAGAACTTCGACCA
GAGGCCAAGAATTTTAAAACCTTTCTTTGTCCACACGCCTAACAGGACG
TATTATCTGATGGACCCAGCGGGAACGCACACAAGTGGTGCAGGAAG
ATCCAGGAGGTTTGGAGGCAGCGATACCAGAGCCACCCGGACGCCGCT
GTGCAGTGA