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## Standard Operating Procedure

### Preparation of active PRK2 [501 - 984]

<b><u>Enzyme description:-</u></b>	PRK2 [501 - 984]
<b><u>Clone number:-</u></b>	DU 1658
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6) and FLAG
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	1-3 mg/L
<b><u>Calculated molecular mass:-</u></b>	59, 739 daltons
<b><u>Purity:-</u></b>	> 90%
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	
	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.
<b><u>Storage temperature:-</u></b>	-70 °C
<b><u>Assay:-</u></b>	Standard filter binding assay
<b><u>Assay buffer:-</u></b>	
	50 mM Tris-HCl pH 7.5, 0.1% 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc
<b><u>Substrate:-</u></b>	
	Long S6 peptide (KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK) Final concentration: 30 μM
<b><u>Specific activity range:-</u></b>	100 - 200 U/mg

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## CLONE DATA SHEET - PRK2 [501 – 984]

**Protein** PRK2 [501 – 984]

**Clone number** DU 1658

**Species** Human

**Accession number** S75548

**Tags** N-terminal His(6) + FLAG (DYKDDDDK)

**Baculovirus expressed protein**

MSYYHHHHHDYDIPTTENLYFQGAMGSATMDYKDDDDK**GKTFLRAPQM**  
**NINIATWGR**LVRRAIPTVNHSGTF**SPQAPVPTTVPVVDVRI**PQLAPPAS  
**DSTVTKLDFDLEPEPPPAPPRASSLGEIDESSEL**RVLDIPGQDSETVFD  
**IQNDRNSILPKSQSEYKPDTPQSGLEYS**GIQELEDRRSQORFQFNLODF  
**RCCA**VLGRGHFGKVLLAEYKNTNEMFAIKALKKGDIVARDEVDSL**MCEK**  
**RIFETVNSVRHPFLVNL**FACFQ**TKEHVCFVMEYAAGGDLMMHI**HTDVFS  
**EPRAV**FYAACVVLGLQYLHEHKIVYRDLKLDNLLDTEGFVKIAD**FGLC**  
**KEGM**GYGDR**TSTFCGTPEFLA**PEVLTETSYTRAVDWWGLGVLIY**EMLVG**  
**ESPF**PGDDEEEV**FDSIVN**DEVRYPRFL**STEAISIMRRLRRN**PER**RLGA**  
**SEKDAEDVKKHPFFRLID**WSALMDKKVK**PPFIPTIRGREDVSN**FDDE**FT**  
**SEAPILTPPREPRILSEEEQEMFRDFDYIADWC**

**Native sequence** Amino acids G501 – C984 (end) of human PRK2.  
Residue G40 of the fusion protein is equivalent to G501 of the native enzyme. The His(6) tag is located at residues 5 - 10 and the FLAG tag at residues 32 – 39.

**Protease cleavage** rTEV site (ENLYFQG) residues 18 – 24

**Cloning sites** BamH1 and EcoR1 site of pFastBAC HTb

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## Nucleotide sequence of insert

GGATCCGCCACCATGGACTACAAGGACGACGATGACAAGGGC  
AAAACATTTCTCAGAGCTCCTCAAATGAATATTAATATTGCC  
ACTTGGGGAAGGCTAGTAAGAAGAGCTATTCTACAGTAAAT  
CATTCTGGCACCTTCAGCCCTCAAGCTCCTGTGCCTACTACA  
GTGCCAGTGGTTGATGTACGCATCCCTCAACTAGCACCTCCA  
GCTAGTGATTCTACAGTAACCAAATTGGACTTTGATCTTGAG  
CCTGAACCTCCTCCAGCCCCACCACGAGCTTCTTCTCTTGGA  
GAAATAGATGAATCTTCTGAATTAAGAGTTTTTGATATACCA  
GGACAGGATTCAGAGACTGTTTTTGATATTCAGAATGACAGA  
AATAGTATACTTCCAAAATCTCAATCTGAATACAAGCCTGAT  
ACTCCTCAGTCAGGCCTAGAATATAGTGGTATTCAGAAGCTT  
GAGGACAGAAGATCTCAGCAAAGTTTTAGTTAATCTACAA  
GATTTTCAGGTGTTGTGCTGTCTTGGGAAGAGGACATTTTGGA  
AAGGTGCTTTTTAGCTGAATATAAAAACACAAATGAGATGTTT  
GCTATAAAAGCCTTAAAGAAAGGAGATATTGTGGCTCGAGAT  
GAAGTAGACAGCCTGATGTGTGAAAAAGAATTTTTGAACT  
GTGAATAGTGTAAGGCATCCCTTTTTGGTGAACCTTTTTGCA  
TGTTTCAAACCAAAGAGCATGTTTGCTTTGTAATGGAATAT  
GCTGCCGGTGGGGACCTAATGATGCACATTCATACTGATGTC  
TTTTCTGAACCAAGAGCTGTATTTTATGCTGCTTGTGTAGTT  
CTTGGGTTGCAGTATTTACATGAACACAAAATTGTTTATAGA  
GATTTGAAATTGGATAACTTATTGCTAGATACAGAGGGCTTT  
GTGAAAATTGCTGATTTTGGTCTTTGCAAAGAAGGAATGGGA  
TATGGAGATAGAACAAGCACATTTTGTGGCACTCCTGAATTT  
CTTGCCCCAGAAGTATTAACAGAACTTCTTATACAAGGGCT  
GTAGATTGGTGGGGCCTTGGCGTGCTTATATATGAAATGCTT  
GTTGGTGAGTCTCCCTTTCCTGGTGATGATGAAGAGGAAGTT  
TTTGACAGTATTGTAAATGATGAAGTAAGGTATCCAAGGTTT  
TTATCTACAGAAGCCATTTCTATAATGAGAAGGCTGTTAAGA  
AGAAATCCTGAACGGCGCCTTGGGGCTAGCGAGAAAGATGCA  
GAGGATGTAAAAAAGCACCCATTTTTCCGGCTAATTGATTGG  
AGCGCTCTGATGGACAAAAAAGTAAAGCCACCATTTATACCT  
ACCATAAGAGGACGAGAAGATGTTAGTAATTTTGATGATGAA  
TTTACCTCAGAAGCACCTATTCTGACTCCACCTCGAGAACCA  
AGGATACTTTCGGAAGAGGAGCAGGAAATGTTTCAGAGATTTT  
GACTACATTGCTGATTGGTGTtaagaattc