

## Standard Operating Procedure

### Preparation of active PLK1 [1 - 603]

<b><u>Enzyme description:-</u></b>	PLK1 [1 - 603]
<b><u>Clone number:-</u></b>	DU 3482
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system Following expression the culture is incubated with 100nM okadaic acid for 3.5 hours prior to purification.
<b><u>Tag:-</u></b>	N-terminal His(6)
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	3 – 5 mg/L

#### **Calculated molecular mass:-**

Monoisotopic            69, 164.98 daltons  
Average Mass            69, 208.82 daltons  
[cysteines reduced, methionines have not been oxidised]

<b><u>Theoretical pI:-</u></b>	9.09
<b><u>Purity:-</u></b>	> 85 %
<b><u>Activation protocol:-</u></b>	Constitutively active

#### **Enzyme storage buffer:-**

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

#### **Storage temperature:-**

-70 °C [Long term stability to be determined]

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**Assay:-**

Standard filter binding assay

**Assay buffer:-**

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

**Substrate:-**

[ISDELMDATFADQEAKKK] – Derived from CDC25 sequence

Final concentration: 300  $\mu$ M

**Specific activity range:-**

To be determined

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## Clone Data Sheet - PLK1 [1 - 603]

**Protein** PLK1 [1 - 603]

**Clone number** DU 3482

**Species** Human

**Accession number** NM\_005030

**Tags** N-terminal His(6)

**Baculovirus expressed protein**  
MHHHHHMSAAVTAGKLARAPADPGKAGVPGV  
AAPGAPAAAPPAKEIPEVLVDPRSRRRYVRGRFLGK  
GGFAKCFEISDADTKEVFAGKIVPKSLLKPHQREK  
MSMEISIHRS LAHQHVVG FHGFFEDNDFV FVVLEL  
CRRRSLELHKRRKALTEPEARYYLRQIVLGCQYLH  
RNRVIHRDLKLG NLFNEDLEV KIGDFGLATKVEY  
DGERKKTLCGTPNYIAPEVLSKKGHSFEVDVWSIG  
CIMYTLLVGKPPFETSCLKETYLR IKKNEYSIPKHINP  
VAASLIQKMLQTDPTARPTINELLNDEFFTSGYIPAR  
LPITCLTIPPRFSIAPSSLDPSNRKPLTVLNKGLENPLP  
ERPREEEPVVRETGEVVDCHLSDMLQQLHSVNAS  
KPSEGLVRQEEAEDPACIPIFWVSKWVDYSDKYGL  
GYQLCDNSVGVLFNDSTRLLILYNDGDSLQYIERDG  
TESYLTVSSH PNSLMKKITLLKYFRNYMSEHLLKAG  
ANITPREGDELARLPYLRTWFRTRSAILHLSNGSVQ  
INFFQDHTKLILCPLMAAVTYIDEKRDFRTYRLSLE  
EYGCKELASRLRYARTMVDKLLSSRSASNRLKAS

**Native sequence** Amino acids M1 – S603 (end) of human PLK1.  
Residue M8 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.

**Protease cleavage** None

**Cloning sites** *Nde*1 and *Xho*1 site in pFastBAC modified

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**Nucleotide  
sequence of insert**

CATATGAGTGCTGCAGTACTGCAGGGAAGCTGGC  
ACGGGCACCGGCCGACCCTGGGAAAGCCGGGGTC  
CCCGGAGTTGCAGCTCCCGGAGCTCCGGCGGCGGC  
TCCACCGGCGAAAGAGATCCCGGAGGTCCTAGTG  
GACCCACGCAGCCGGCGGCGCTATGTGCGGGGCC  
GCTTTTGGGCAAGGGCGGCTTTGCCAAGTGCTTC  
GAGATCTCGGACGCGGACACCAAGGAGGTGTTCCG  
CGGGCAAGATTGTGCCTAAGTCTCTGCTGCTCAAG  
CCGCACCAGAGGGAGAAGATGTCCATGAAATAT  
CCATTCACCGCAGCCTCGCCCACCAGCACGTCGTA  
GGATTCCACGGCTTTTTCGAGGACAACGACTTCGT  
GTTTCGTGGTGTGGAGCTCTGCCGCCGGAGGTCTCT  
CCTGGAGCTGCACAAGAGGAGGAAAGCCCTGACT  
GAGCCTGAGGCCCATACTACCTACGGCAAATTGT  
GCTTGGCTGCCAGTACCTGCACCGAAACCGAGTTA  
TTCATCGAGACCTCAAGCTGGGCAACCTTTTCTCTG  
AATGAAGATCTGGAGGTGAAAATAGGGGATTTTG  
GACTGGCAACCAAAGTCGAATATGACGGGGAGAG  
GAAGAAGACCCTGTGTGGGACTCCTAATTACATAG  
CTCCCGAGGTGCTGAGCAAGAAAGGGCACAGTTT  
CGAGGTGGATGTGTGGTCCATTGGGTGTATCATGT  
ATACCTTGTTAGTGGGCAAACCACCTTTTGAGACTT  
CTTGCCTAAAAGAGACCTACCTCCGGATCAAGAA  
GAATGAATACAGTATTCCCAAGCACATCAACCCC  
GTGGCCGCCTCCCTCATCCAGAAGATGCTTCAGAC  
AGATCCCCTGCCCCGCCAACCATTAACGAGCTGC  
TTAATGACGAGTTCTTTACTTCTGGCTATATCCCTG  
CCCGTCTCCCCATCACCTGCCTGACCATTCCACCA  
AGGTTTTTCGATTGCTCCCAGCAGCCTGGACCCAG  
CAACCGGAAGCCCCTCACAGTCCTCAATAAAGGC  
TTGGAGAACCCCTGCCTGAGCGTCCCCGGGAAA  
AAGAAGAACCAGTGGTTCGAGAGACAGGTGAGGT  
GGTCGACTGCCACCTCAGTGACATGCTGCAGCAGC  
TGCACAGTGTCAATGCCTCCAAGCCCTCGGAGCGT  
GGGCTGGTCAGGCAAGAGGAGGCTGAGGATCCTG  
CCTGCATCCCCATCTTCTGGGTGAGCAAGTGGGTG  
GACTATTCGGACAAGTACGGCCTTGGGTATCAGCT  
CTGTGATAACAGCGTGGGGGTGCTCTTCAATGACT  
CAACACGCCTCATCCTCTACAATGATGGTGACAGC  
CTGCAGTACATAGAGCGTGACGGCACTGAGTCCTA  
CCTCACCGTGAGTTCCCATCCCAACTCCTTGATGA  
AGAAGATCACCTCCTTAAATATTTCCGCAATTAC

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ATGAGCGAGCACTTGCTGAAGGCAGGTGCCAACA  
TCACGCCGCGCGAAGGTGATGAGCTCGCCCGGCTG  
CCCTACCTACGGACCTGGTTCCGCACCCGCAGCGC  
CATCATCCTGCACCTCAGCAACGGCAGCGTGCAGA  
TCAACTTCTTCCAGGATCACACCAAGCTCATCTTGT  
GCCCACTGATGGCAGCCGTGACCTACATCGACGA  
GAAGCGGGACTTCCGCACATAACCGCCTGAGTCTCC  
TGGAGGAGTACGGCTGCTGCAAGGAGCTGGCCAG  
CCGGCTCCGCTACGCCCGCACTATGGTGGACAAGC  
TGCTGAGCTCACGCTCGGCCAGCAACCGTCTCAAG  
GCCTCCTAACTCGAG