Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PLK1 [1 - 603]

Enzyme description:- PLK1 [1 - 603]

Clone number:- DU 3482

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:-

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

Theoretical pI:- 9.09

Purity:- > 85 %

Activation protocol:-

PLK1 (2.5 µM) is activated by incubation with 100 µg/ml GST-MST2 [DU 1433] in 50mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP for 30 min at 30 °C. Following activation, PLK1 cannot be removed from the MST2.

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 µM

Specific activity range:- To be determined

Division of Signal Transduction Therapy

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	MHHHHHH**MSAAVTAGKLARAPADPGKAGVPGVAAPGAPAAAPPAKEIPE VLVDPRSRRRYVRGRFLGKGGFAKCFEISDADTKEVFAGKIVPKSLLLK PHQREKMSMEISIHRSLAHQHVVGFHGFFEDNDFVFVVLELCRRRSLLE LHKRRKALTEPEARYYLRQIVLGCQYLHRNRVIHRDLKLGNLFLNEDLE VKIGDFGLATKVEYDGERKKTLCGTPNYIAPEVLSKKGHSFEVDVWSIG CIMYTLLVGKPPFETSCLKETYLRIKKNEYSIPKHINPVAASLIQKMLQ TDPTARPTINELLNDEFFTSGYIPARLPITCLTIPPRFSIAPSSLDPSN RKPLTVLNKGLENPLPERPREKEEPVVRETGEVVDCHLSDMLQQLHSVN ASKPSERGLVRQEEAEDPACIPIFWVSKWVDYSDKYGLGYQLCDNSVGV LFNDSTRLILYNDGDSLQYIERDGTESYLTVSSHPNSLMKKITLLKYFR NYMSEHLLKAGANITPREGDELARLPYLRTWFRTRSAIILHLSNGSVQI NFFQDHTKLILCPLMAAVTYIDEKRDFRTYRLSLLEEYGCCKELASRLR YARTMVDKLLSSRSASNRLKAS**
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*1site in pFastBAC modified

Nucleotide sequence of insert

catATGAGTGCTGCAGTGACTGCAGGGAAGCTGGCACGGGCACCGGCCG
ACCCTGGGAAAGCCGGGGTCCCCGGAGTTGCAGCTCCCGGAGCTCCGGC
GGCGGCTCCACCGGCGAAAGAGATCCCGGAGGTCCTAGTGGACCCACGC
AGCCGGCGGCGCTATGTGCGGGGCCGCTTTTTGGGCAAGGGCGGCTTTG
CCAAGTGCTTCGAGATCTCGGACGCGGACACCAAGGAGGTGTTCGCGGG
CAAGATTGTGCCTAAGTCTCTGCTGCTCAAGCCGCACCAGAGGGAGAAG
ATGTCCATGGAAATATCCATTCACCGCAGCCTCGCCCACCAGCACGTCG
TAGGATTCCACGGCTTTTTCGAGGACAACGACTTCGTGTTCGTGGTGTT
GGAGCTCTGCCGCCGGAGGTCTCTCCTGGAGCTGCACAAGAGGAGGAAA
GCCCTGACTGAGCCTGAGGCCCGATACTACCTACGGCAAATTGTGCTTG
GCTGCCAGTACCTGCACCGAAACCGAGTTATTCATCGAGACCTCAAGCT
GGGCAACCTTTTCCTGAATGAAGATCTGGAGGTGAAAATAGGGGATTTT
GGACTGGCAACCAAAGTCGAATATGACGGGGAGAGGAAGAAGACCCTGT
GTGGGACTCCTAATTACATAGCTCCCGAGGTGCTGAGCAAGAAAGGGCA
CAGTTTCGAGGTGGATGTGTGGTCCATTGGGTGTATCATGTATACCTTG
TTAGTGGGCAAACCACCTTTTGAGACTTCTTGCCTAAAAGAGACCTACC
TCCGGATCAAGAAGAATGAATACAGTATTCCCAAGCACATCAACCCCGT
GGCCGCCTCCCTCATCCAGAAGATGCTTCAGACAGATCCCACTGCCCGC
CCAACCATTAACGAGCTGCTTAATGACGAGTTCTTTACTTCTGGCTATA
TCCCTGCCCGTCTCCCCATCACCTGCCTGACCATTCCACCAAGGTTTTC
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CTCAATAAAGGCTTGGAGAACCCCCTGCCTGAGCGTCCCCGGGAAAAAG
AAGAACCAGTGGTTCGAGAGACAGGTGAGGTGGTCGACTGCCACCTCAG
TGACATGCTGCAGCAGCTGCACAGTGTCAATGCCTCCAAGCCCTCGGAG
CGTGGGCTGGTCAGGCAAGAGGAGGCTGAGGATCCTGCCTGCATCCCCA
TCTTCTGGGTCAGCAAGTGGGTGGACTATTCGGACAAGTACGGCCTTGG
GTATCAGCTCTGTGATAACAGCGTGGGGGTGCTCTTCAATGACTCAACA
CGCCTCATCCTCTACAATGATGGTGACAGCCTGCAGTACATAGAGCGTG
ACGGCACTGAGTCCTACCTCACCGTGAGTTCCCATCCCAACTCCTTGAT
GAAGAAGATCACCCTCCTTAAATATTTCCGCAATTACATGAGCGAGCAC
TTGCTGAAGGCAGGTGCCAACATCACGCCGCGCGAAGGTGATGAGCTCG
CCCGGCTGCCCTACCTACGGACCTGGTTCCGCACCCGCAGCGCCATCAT
CCTGCACCTCAGCAACGGCAGCGTGCAGATCAACTTCTTCCAGGATCAC
ACCAAGCTCATCTTGTGCCCACTGATGGCAGCCGTGACCTACATCGACG
AGAAGCGGGACTTCCGCACATACCGCCTGAGTCTCCTGGAGGAGTACGG
CTGCTGCAAGGAGCTGGCCAGCCGGCTCCGCTACGCCCGCACTATGGTG
GACAAGCTGCTGAGCTCACGCTCGGCCAGCAACCGTCTCAAGGCCTCCt
aactcgag