Standard Operating Procedure

Preparation of PLK1 [37 - 338]

Enzyme description:- PLK1 [37 - 338]

Clone number:- DU 61680

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-
Monoisotopic 61, 361.92 daltons
Average Mass 61, 401.41 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.54

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
ISDELMDATFADQEAKKK – Derived from CDC25 sequence
Final concentration: 300 µM

Clone Data Sheet

PLK1 [37 - 338]

Protein	PLK1 [37 - 338]
Clone number	DU 61680
Species	Human
Accession number	NM_005030.6
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PPAKEIPEVLVDPR** **SRRRYVRGRFLGKGGFAKCFEISDADTKEVFAGKIVPKSLLLKPHQREK** **MSMEISIHRSLAHQHVVGFHGFFEDNDFVFVVLELCRRRSLLELHKRRK** **ALTEPEARYYLRQIVLGCQYLHRNRVIHRDLKLGNLFLNEDLEVKIGDF** **GLATKVEYDGERKKTLCGTPNYIAPEVLSKKGHSFEVDVWSIGCIMYTL** **LVGKPPFETSCLKETYLRIKKNEYSIPKHINPVAASLIQKMLQTDPTAR** **PTINELLNDEFFTSGYIPARLPITCLTIPPRFSIAPSSLDPSN**
Native sequence	Amino acids P37 – N338 (end residue is S603) of human PLK1. Residue P232 of the fusion protein is equivalent to P37 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

ggatccCCACCGGCGAAAGAGATCCCGGAGGTCCTAGTGGACCCACGCA
GCCGGCGGCGCTATGTGCGGGGCCGCTTTTTGGGCAAGGGCGGCTTTGC
CAAGTGCTTCGAGATCTCGGACGCGGACACCAAGGAGGTGTTCGCGGGC
AAGATTGTGCCTAAGTCTCTGCTGCTCAAGCCGCACCAGAGGGAGAAGA
TGTCCATGGAAATATCCATTCACCGCAGCCTCGCCCACCAGCACGTCGT
AGGATTCCACGGCTTTTTCGAGGACAACGACTTCGTGTTCGTGGTGTTG
GAGCTCTGCCGCCGGAGGTCTCTCCTGGAGCTGCACAAGAGGAGGAAAG
CCCTGACTGAGCCTGAGGCCCGATACTACCTACGGCAAATTGTGCTTGG
CTGCCAGTACCTGCACCGAAACCGAGTTATTCATCGAGACCTCAAGCTG
GGCAACCTTTTCCTGAATGAAGATCTGGAGGTGAAAATAGGGGATTTTG
GACTGGCAACCAAAGTCGAATATGACGGGGAGAGGAAGAAGACCCTGTG
TGGGACTCCTAATTACATAGCTCCCGAGGTGCTGAGCAAGAAAGGGCAC
AGTTTCGAGGTGGATGTGTGGTCCATTGGGTGTATCATGTATACCTTGT
TAGTGGGCAAACCACCTTTTGAGACTTCTTGCCTAAAAGAGACCTACCT
CCGGATCAAGAAGAATGAATACAGTATTCCCAAGCACATCAACCCCGTG
GCCGCCTCCCTCATCCAGAAGATGCTTCAGACAGATCCCACTGCCCGCC
CAACCATTAACGAGCTGCTTAATGACGAGTTCTTTACTTCTGGCTATAT
CCCTGCCCGTCTCCCCATCACCTGCCTGACCATTCCACCAAGGTTTTCG
ATTGCTCCCAGCAGCCTGGACCCCAGCAACtgagcggccgc