

MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of PLK4 [1 - 269]

Enzyme description:- PLK4 [1 - 269]

Clone number:- DU 62994

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 57, 562.27 daltons

Average Mass 57, 599.85 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.71

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:-

Myelin Basic protein Final concentration: 1 mg/ml

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Clone Data Sheet

PLK4 [1 - 269]

<u>Protein</u>	PLK4 [1 - 269]
<u>Clone number</u>	DU 62994
<u>Species</u>	Human
<u>Accession number</u>	NM_014264.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMATCIGEKIEDFKV GNLLGKGSFAGVYRAESIHTGLEVAIKMIDKKAMYKAGMVQRVQNEVKI HCQLKHPSILELYNYFEDSNYVYLVLEMCHNGEMNRYLKNRVKPFSENE ARHFMHQIITGMLYLHSHGILHRDLTLSNLLLTRNMNIKIADFGLATQL KMPHEKHYTLCGTPNYISPEIATRSAHGLESDVWSLGCMFYTL LI GRPP FDTDTVKNTLNKVVLADYEMPSFLSIEAKDLIHQLLRNPADRLSLSSV LDHPFMSRNS
<u>Native sequence</u>	Amino acids M1 – S269 (end residue is H970) of human PLK4. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence of Insert

ggatccATGGCGACCTGCATCGGGGAGAAGATCGAGGATTTTAAAGTTGGAAATCTGCTTGGTAAA
GGATCATTTGCTGGTGTCTACAGAGCTGAGTCCATTCACACTGGTTTGGGAAGTTGCAATCAAATG
ATAGATAAGAAAAGCCATGTACAAAGCAGGAATGGTACAGAGAGTCCAAAATGAGGTGAAAATACAT
TGCCAATTGAAACATCCTTCTATCTTGGAGCTTTATAACTATTTTGAAGATAGCAATTATGTGTAT
CTGGTATTAGAAATGTGCCATAATGGAGAAATGAACAGGTATCTAAAGAATAGAGTGAAACCCTTC
TCAGAAAATGAAGCTCGACACTTCATGCACCAGATCATCACAGGGATGTTGTATCTTCATTCTCAT
GGTATACTACACCGGGACCTCACACTTTCTAACCTCCTACTGACTCGTAATATGAACATCAAGATT
GCTGATTTTGGGCTGGCAACTCAACTGAAAATGCCACATGAAAAGCACTATACATTATGTGGAAC
CCTAACTACATTTACCAGAAATGCCACTCGAAGTGCACATGGCCTTGAATCTGATGTTTGGTCC
CTGGGCTGTATGTTTTATACATTACTTATCGGGAGACCACCTTCGACACTGACACAGTCAAGAAC
ACATTAAATAAAGTAGTATTGGCAGATTATGAAATGCCATCTTTTTTGTCAATAGAGGCCAAGGAC
CTTATTCACCAGTTACTTCGTAGAAATCCAGCAGATCGTTTAAAGTCTGTCTTCAGTATTGGACCAT
CCTTTTATGTCCCGAAATTCTtgagcggccgc

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