

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PLK3 [1 - 646]

<u>Enzyme description:-</u>	PLK3 [1 - 646]
<u>Clone number:-</u>	DU 35183
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 98, 390.86 daltons
Average Mass 98, 453.49 daltons
[cysteines reduced, methionines have not been oxidized]

Theoretical pI:- 8.76

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, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

ISDELMDATFADQEAKKK

Final concentration: 300 uM

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Clone Data Sheet

PLK3 [1 - 646]

<u>Protein</u>	PLK3 [1 - 646]
<u>Clone number</u>	DU 35183
<u>Species</u>	Human
<u>Accession number</u>	NM_004073.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMEPAAAGFLSPRPFQ RAAAPAPPAGPGPPPSALRGPELEMLAGLPTSDPGRLITDPRSGRTYL KGRLLGKGGFARCYEATDTETGSAYAVKVIPQSRVAKPHQREKILNEIE LHRDLQHRHIVRFSHHFEDADNIYIFLELCSRKSLAHIWKARHTLLEPE VRYYLRQILSGLKYLHQRGILHRDLKLGNNFFITENMELKVGDFGLAARL EPPEQRKKTICGTPNYVAPEVLLRQGHGPEADVWSLGCVMYTLLCGSP FETADLKETYRCIKQVHYTLPASLSLPAEQLLAAILRASPRDRPSIDQI LRHDFFTKGYTPDRLPISSCVTVPDLTTPNPARS LF AKVTKSLFGRKKK SKNHAQERDEVSGLVSGLMRTSVGHQDARPEAPAASGPAPVSLVETAPE DSSPRGTLASSGDGFEEGLTVATVVESALCALRN CIAFMPPAEQNPA PL AQPEPLVWVSKWVDYSNKFGFGYQLSSRRVAVLFNDGTHMAL SANRKT V HYNPTSTKHFSFSVGAVPRALQPQLGILRYFASYMEQHLMKGGDLPSVE EVEVPAPPLLQWVKTDQALLMLFSDGTVQVNFYGDHTKLILSGWEPLL VTFVARNRSACTYLASHLRQLGCSPDLRQRLRYALRLLRDRSPA
<u>Native sequence</u>	Amino acids M1 – A646 (end) of human PLK3. 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 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGGAGCCTGCCGCCGGTTTCCTGTCTCCGCGCCCCCTTCCAGC
GTGCGGCCGCGCGCCCCGCTCCCCCGGCCGGGCCCGGGCCGCTCCGAG
TGCCTTGCGCGGACCTGAGCTGGAGATGCTGGCCGGGCTACCGACGTCA
GACCCCGGGCGCCTCATCACGGACCCGCGCAGCGGGCCGCACCTACCTCA
AAGGCCGCTTGTTGGGCAAGGGGGGCTTCGCCCGCTGCTACGAGGCCAC
TGACACAGAGACTGGCAGCGCCTACGCTGTCAAAGTCATCCCGCAGAGC
CGCGTCGCCAAGCCGCATCAGCGCGAGAAGATCCTAAATGAGATTGAGC
TGCACCGAGACCTGCAGCACCGCCACATCGTGCGTTTTTTCGCACCACTT
TGAGGACGCTGACAACATCTACATTTTCTTGAGCTCTGCAGCCGAAAG
TCCCTGGCCCACATCTGGAAGGCCCGGCACACCCTGTTGGAGCCAGAAG
TGCGCTACTACCTGCGGCAGATCCTTTCTGGCCTCAAGTACTTGCACCA
GCGCGGCATCTTGACCCGGGACCTCAAGTTGGGAAATTTTTTCATCACT
GAGAACATGGAAGTGAAGGTGGGGGATTTTGGGCTGGCAGCCCGGTTGG
AGCCTCCGGAGCAGAGGAAGAAGACCATCTGTGGCACCCCCAACTATGT
GGCTCCAGAAGTGCTGCTGAGACAGGGCCACGGCCCTGAGGCGGATGTA
TGGTCACTGGGCTGTGTATGTACACGCTGCTCTGCGGGAGCCCTCCCT
TTGAGACGGCTGACCTGAAGGAGACGTACCGCTGCATCAAGCAGGTTCA
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GCCATCCTTCGGGCCTCACCCCGAGACCGCCCCCTCTATTGACCAGATCC
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CAGCAGCTGCGTGACAGTCCCAGACCTGACACCCCCCAACCCAGCTAGG
AGTCTGTTTGCCAAAGTTACCAAGAGCCTCTTTGGCAGAAAGAAGAAGA
GTAAGAATCATGCCCAGGAGAGGGATGAGGTCTCCGGTTTGGTGAGCGG
CCTCATGCGCACATCCGTTGGCCATCAGGATGCCAGGCCAGAGGCTCCA
GCAGCTTCTGGCCCAGCCCCTGTCAGCCTGGTAGAGACAGCACCTGAAG
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CCCAGCCAGAGCCTCTGGTGTGGGTCAGCAAGTGGGTTGACTACTCCAA
TAAGTTCGGCTTTGGGTATCAACTGTCCAGCCGCCGTGTGGCTGTGCTC
TTCAACGATGGCACACATATGGCCCTGTCGGCCAACAGAAAGACTGTGC
ACTACAATCCCACCAGCACAAAGCACTTCTCCTTCTCCGTGGGTGCTGT
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TCAGGCTCTCCTCATGCTGTTTAGTGATGGCACTGTCCAGGTGAACTTC
TACGGGGACCACACCAAGCTGATTCTCAGTGGCTGGGAGCCCCCTCCTTG
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CCTTCGGCAGCTGGGCTGCTCTCCAGACCTGCGGCAGCGACTCCGCTAT
GCTCTGCGCCTGCTCCGGGACCGCAGCCCAGCCtagggatcc