Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Cyclin K [11 - 267]

Enzyme description:- Cyclin K [11 - 4267]

Clone number:- DU 46325

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 56, 971.96 daltons
Average Mass 57, 009.04 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.30

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

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Clone Data Sheet

Cyclin K [11 - 267]

Protein	Cyclin K [11 - 267]
Clone number	DU 46325
Species	Human
Accession number	NM_001099402.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SVTSANLDHTKPCW** **YWDKKDLAHTPSQLEGLDPATEARYRREGARFIFDVGTRLGLHYDTLAT** **GIIYFHRFYMFHSFKQFPRYVTGACCLFLAGKVEETPKKCKDIIKTARS** **LLNDVQFGQFGDDPKEEVMVLERILLQTIKFDLQVEHPYQFLLKYAKQL** **KGDKNKIQKLVQMAWTFVNDSLCTTLSLQWEPEIIAVAVMYLAGRLCKF** **EIQEWTSKPMYRRWWEQFVQDVPVDVLEDICHQILDLYSQGKQQMPH**

Native sequence	Amino acids S11 – H267 (end residue is R580) of human Cyclin K. Residue S232 of the fusion protein is equivalent to S11 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

ggatccTCAGTAACTTCAGCAAACCTGGACCACACAAAGCCATGTTGGTA
CTGGGATAAGAAAGACTTGGCTCATACACCCTCACAACTTGAAGGACTTG
ATCCAGCCACCGAGGCCCGGTACCGCCGAGAGGGCGCTCGGTTCATCTTT
GATGTGGGCACACGTTTGGGGCTACACTATGATACCCTGGCAACTGGAAT
AATTTATTTTCATCGCTTCTATATGTTTCATTCCTTCAAGCAATTCCCAA
GATATGTGACAGGAGCCTGTTGCCTCTTTCTGGCTGGGAAAGTAGAAGAA
ACACCAAAAAAATGTAAAGATATCATCAAAACAGCTCGTAGTTTATTAAA
TGATGTACAATTTGGCCAGTTTGGAGATGACCCAAAGGAGGAAGTAATGG
TTCTGGAGAGAATCTTACTGCAGACCATCAAGTTTGATTTACAGGTAGAA
CATCCATACCAGTTCCTACTAAAATATGCAAAGCAACTCAAAGGTGATAA
AAACAAAATTCAAAAGTTGGTTCAAATGGCATGGACATTTGTAAATGACA
GTCTCTGCACCACCTTGTCACTGCAGTGGGAACCAGAGATCATAGCAGTA
GCAGTGATGTATCTCGCAGGACGTTTGTGCAAATTTGAAATACAAGAATG
GACCTCCAAACCCATGTATAGGAGATGGTGGGAGCAGTTTGTTCAAGATG
TCCCGGTCGACGTTTTGGAAGACATCTGCCACCAAATCCTGGATCTTTAC
TCACAAGGAAAACAACAGATGCCTCATtaagcggccgc