

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active CDK9 [1 – 372] / Cyclin T1 [1 – 726]

Enzyme description:- CDK9 [1 – 372] / Cyclin T1 [1 – 726]

Clone number:- DU 31050

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST for CDK9

Purification method:- Glutathione Sepharose

Calculated molecular mass:-

Monoisotopic 69, 556.79 daltons (CDK9)
 80, 634.49 daltons (Cyclin T1)

Average Mass 69, 601.69 daltons (CDK9)
 80, 684.62 daltons (Cyclin T1)

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.1 GST-CDK9 and 8.9 Cyclin T1

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA,
0.1 mM sodium vanadate, 10 mM magnesium acetate

Substrate:-

YSPTSPSYPTSPSYPTSPSKKK Final concentration: 300 μ M

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Clone Data Sheet

CDK9 [1 – 372] and Cyclin T1 [1 - 726]

<u>Protein</u>	CDK9 [1 – 372] and Cyclin T1 [1 - 726]
<u>Clone number</u>	DU 31050
<u>Species</u>	Human
<u>Accession number</u>	NM_001261 [CDK9] and NM_001240.2 [Cyclin T1]
<u>Tags</u>	N-terminal GST (CDK9)
<u>Insect cell expressed CDK9</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKL TQSM A I RYIADKHNLGGCPKERA E ISMLE GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDP MCLDAFPKLVCFKKRIE AIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGMAKQYDSVE CPFCDEVSKYEKLAKIGQGT FGEVFKARHRKTGQKVALKKVLMENEKE GFPITALREIKILQLLKHENVVNLIEICRTKASPNRCKGSIYLVFDF CEHDLAGLLSNVLVKFTLSEIKRVMQMLLNGLYIHRNKILHRDMKAA NVLI TRDGV LKLADFGLARAFSLAKNSQPNRYTNRVVTLWYRPELLL GERDYGPPIDLWGAGCIMAEMWTRSPIMQGNTEQHQLALISQLCGSIT PEVWPVNDNYELYEKLELVKGQKRKVKDRLKAYVRDPYALDLIDKLLV LDPAQRIDSDDALNHDFFWSDPMPSDLKGMLSTHLTSMFEYLAPPRK GSQITQOSTNQSRNPATTNQTEFERVF</p>
<u>Native sequence</u>	Amino acids M1 – F372 (end) of human CDK9. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located between residues 1- 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229

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Insect cell
expressed Cyclin
T1

MEGERKNNNKRWYFTREQLNSPSRRFGVDPDKELSYRQQAANLLQDM
GQRLNVSQLTINTAIVYMHRFYMIQSFTQFPGNSVAPAALFLAAKVEE
QPKKLEHVIKVAHTCLHPQESLPDTRSEAYLQQVQDLVILESIILOTL
GFELTIDHPHTHVVKCTQLVRASKDLAQTSYFMATNSLHLTTFSLQYT
PPVVACVCIHLACKWSNWEIPVSTDGKHWWEYVDATVTLELLDELTHE
FLQILEKTPNRLKRIWNWRACEAAKKTADDRGTDEKTSEQTILNMIS
QSSSDTTIAGLMSMSTSTTSVAVPSLPVSEESSNLTSVEMLPGKRWLS
SQPSFKLEPTQGHRTSENLALTGVDHSLPQDGSNAFISQKQNSKSVPS
AKVSLKEYRAKHAEEELAAQKQLENMEANVKSQYAYAAQNLLSHHDSH
SSVILKMPIEGSENPERPFLEKADKTALKMRI PVAGGDKAASSKPEEI
KMRIKVHAAADKHNSVEDSVTKSREHKEKHKTHPSNHHHHHHNHSHKH
SHSQLPVGTGNKRPGDPKHSSQTSNLAHKTYLSLSSSFSSSSSTRKRGP
SEETGGAVFDHPAKIAKSTKSSSLNFSFPSLPTMGQMPGHSSDTSGLS
FSQPSCKTRVPHSKLDKGPTGANGHNTTQIDYQDTVNMLHSLLSAQG
VQPTQPTAFEFVRPYSDYLNPRSGGISSRSGNTDKPRPPPLPSEPPPP
LPPLPK

Native sequence

Amino acids M1 – K726 (end) of human cyclin T1.

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Nucleotide sequence of CDK9 insert

ggatccATGGCAAAGCAGTACGACTCGGTGGAGTGCCCTTTTTGTGAT
GAAGTTTCCAAATACGAGAAGCTCGCCAAGATCGGCCAAGGCACCTTC
GGGAGGTGTTCAAGGCCAGGCACCGCAAGACCGGCCAGAAGGTGGCT
CTGAAGAAGGTGCTGATGGAAAACGAGAAGGAGGGTTCCCCATTACA
GCCTTGCGGGAGATCAAGATCCTTCAGCTTCTAAACACGAGAATGTG
GTCAACTTGATTGAGATTTGTGCAACCAAAGCTTCCCCCTATAACCGC
TGCAAGGGTAGTATATACTGGTGTTCGACTTCTGCGAGCATGACCTT
GCTGGGCTGTTGAGCAATGTTTTGGTCAAGTTCACGCTGTCTGAGATC
AAGAGGGTGATGCAGATGCTGCTTAACGGCCTCTACTACATCCACAGA
ACAAGATCCTGCATAGGGACATGAAGGCTGCTAATGTGCTTATCACT
CGTGATGGGGTCCCTGAAGCTGGCAGACTTTGGGCTGGCCCGGGCCTTC
AGCCTGGCCAAGAACAGCCAGCCCAACCGCTACACCAACCGTGTGGTG
ACACTCTGGTACCGGCCCCCGGAGCTGTTGCTCGGGGAGCGGGACTAC
GGCCCCCATTGACCTGTGGGGTGCTGGGTGCATCATGGCAGAGATG
TGGACCCGCAGCCCCATCATGCAGGGCAACACGGAGCAGCACCACCTC
GCCCTCATCAGTCAGCTCTGCGGCTCCATCACCCCTGAGGTGTGGCCA
AACGTGGACAACTATGAGCTGTACGAAAAGCTGGAGCTGGTCAAGGGC
CAGAAGCGGAAGGTGAAGGACAGGCTGAAGGCCTATGTGCGTGACCCA
TACGCACTGGACCTCATCGACAAGCTGCTGGTGCTGGACCCTGCCAG
CGCATCGACAGCGATGACGCCCTCAACCACGACTTCTTCTGGTCCGAC
CCCATGCCCTCCGACCTCAAGGGCATGCTCTCACCCACCTGACGTCC
ATGTTTCGAGTACTTGGCACCACCGCGCCGGAAGGGCAGCCAGATCACC
CAGCAGTCCACCAACCAGAGTCGCAATCCCGCCACCACCAACCAGACG
GAGTTTGAGCGCGTCTTctgaggatcc

Nucleotide sequence of cyclin T1 insert

ccATGGAGGGAGAGAGGAAGAACAACAACAAACGGTGGTATTTCACTC
GAGAACAGCTGGAAAATAGCCCATCCCGTCGTTTTGGCGTGGACCCAG
ATAAAGAACTTTCTTATCGCCAGCAGGCGGCCAATCTGCTTCAGGACA
TGGGGCAGCGTCTTAACGTCTCACAATTGACTATCAACACTGCTATAG
TATACATGCATCGATTCTACATGATTTCAGTCCTTCACACAGTTCCCTG
GAAATTCTGTGGCTCCAGCAGCCTTGTTTCTAGCAGCTAAAGTGGAGG
AGCAGCCCAAAAATTGGAACATGTCATCAAGGTAGCACATACTTGTC
TCCATCCTCAGGAATCCCTTCCCTGATACTAGAAGTGAGGCTTATTTGC
ACAAGTTCAAGATCTGGTCATTTTAGAAAGCATAATTTTGCAGACTT
TAGGCTTTGAACTAACAATTGACCACCCACATACTCATGTAGTAAAGT
GCACTCAACTTGTTTCGAGCAAGCAAGGACTTAGCACAGACTTCTTACT
TCATGGCAACCAACAGCCTGCATTTGACCACATTTAGCCTGCAGTACA
CACCTCCTGTGGTGGCCTGTGTCTGCATTCACCTGGCTTGCAAGTGGT
CCAATTGGGAGATCCAGTCTCAACTGACGGGAAGCACTGGTGGGAGT
ATGTTGACGCCACTGTGACCTTGGAACTTTTAGATGAACTGACACATG
AGTTTCTACAGATTTTGGAGAAAACCTCCAACAGGCTCAAACGCATTT
GGAATTGGAGGGCATGCGAGGCTGCCAAGAAAACAAAAGCAGATGACC
GAGGAACAGATGAAAAGACTTCAGAGCAGACAATCCTCAATATGATTT
CCCAGAGCTCTTCAGACACAACCATTGCAGGTTTAAATGAGCATGTCAA
CTTCTACCACAAGTGCAGTGCCTTCCCTGCCAGTCTCCGAAGAGTCAT
CCAGCAACTTAACCAGTGTGGAGATGTTGCCGGGCAAGCGTTGGCTGT

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CCTCCCAACCTTCTTTCAAACCTAGAACCTACTCAGGGTCATCGGACTA
GTGAGAATTTAGCACTTACAGGAGTTGATCATTCTTACCACAGGATG
GTTCAAATGCATTTATTTCCAGAAAGCAGAATAGTAAGAGTGTGCCAT
CAGCTAAAGTGTCACTGAAAGAATACCGCGCGAAGCATGCAGAAGAAT
TGGCTGCCCAGAAGAGGCAACTGGAGAACATGGAAGCCAATGTGAAGT
CACAATATGCATATGCTGCCCAGAATCTCCTTTCTCATCATGATAGCC
ATTCTTCAGTCATTCTAAAAATGCCCATAGAGGGTTTCAGAAAACCCGG
AGCGGCCTTTTCTGGAAAAGGCTGACAAAACAGCTCTCAAAATGAGAA
TCCCAGTGGCAGGTGGAGATAAAGCTGCGTCTTCAAACCCAGAGGAGA
TAAAAATGCGCATAAAAGTCCATGCTGCAGCTGATAAGCACAATTCTG
TAGAGGACAGTGTTACAAAGAGCCGAGAGCACAAAGAAAAGCACAAGA
CTCACCCATCTAATCATCATCATCATAATCACCCTCACACAAGC
ACTCTCATTCCCAACTTCCAGTTGGTACTGGGAACAAACGTCCTGGTG
ATCCAAAACATAGTAGCCAGACAAGCAACTTAGCACATAAAACCTATA
GCTTGTCTAGTTCTTTTTCTTCCAGTTCTACTCGTAAAAGGGGAC
CCTCTGAAGAGACTGGAGGGGCTGTGTTTGATCATCCAGCCAAGATTG
CCAAGAGTACTAAATCCTCTTCCCTAAATTTCTCCTTCCCTTCACTTC
CTACAATGGGTCAGATGCCTGGGCATAGCTCAGACACAAGTGGCCTTT
CCTTTTACAGCCCAGCTGTAAACTCGTGTCCCTCATTCGAAACTGG
ATAAAGGGCCACTGGGGCCAATGGTCACAACACGACCCAGACAATAG
ACTATCAAGACACTGTGAATATGCTTCACTCCCTGCTCAGTGCCAGG
GTGTTCAAGCCACTCAGCCTACTGCATTTGAATTTGTTTCGTCCTTATA
GTGACTATCTGAATCCTCGGTCTGGTGGAATCTCCTCGAGATCTGGCA
ATACAGACAAACCCCGGCCACCACCTCTGCCATCAGAACCTCCTCCAC
CACTTCCACCCCTTCCTaagtaaggtacc