

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Cyclin T1 [1 - 726]

Enzyme description:- Cyclin T1 [1 - 726]

Clone number:- DU 45926

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 107, 441.13 daltons

Average Mass 107, 508.78 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.23

Purity:- >75 %

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 T1 [1 - 726]

Protein Cyclin T1 [1 - 726]

Clone number DU 45926

Species Human

Accession number NM_001240.3

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL
DIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA
WPLQGWQATFGGGDHPPKSDLEVLFGQPLGSM~~EGERKNNNKRWYFTREQL~~
~~ENSPSRRFGVDPDKELSYRQQAANLLQDMGQRLNVSQLTINTAIVMHRF~~
~~YMIQSFTQFPGNSVAPAALFLAAKVEEQPKKLEHVIKVAHTCLHPQESLP~~
~~DTRSEAYLQQVQDLVILESIILQTLGFELTIDHPHTHVVKCTQLVRASKD~~
~~LAQTSYFMATNSLHLTTFSLQYTPPVVACVCIHLACKWSNWEIPVSTDGK~~
~~HWWEYVDATVTLELLDELTHEFLQILEKTPNRLKRIWNWRACEAAKKTKA~~
~~DDRGTDEKTSEQTILNMISQSSSDTTIAGLSMSTSTTSAPVSLPVSEES~~
~~SSNLTSVEMLPGKRWLSSQPSFKLEPTQGHRTSENLALTGVDHSLPDGGS~~
~~NAFISQKQNSKSVPSAKVSLKEYRAKHAEELAAQKRQLENMEANVKSQYA~~
~~YAAQNLLSHHDSHSSVILKMPIEGSENPERPFLEKADKTALKMRIPVAGG~~
~~DKAASSKPEEIKMRIKVHAAADKHNSVEDSVTKSREHKEKHKTHPSNHHH~~
~~HHNHHSHKSHSQLPVG TGNKRP GPKHSSQTSNLAHKTYLSSSFSSSS~~
~~STRKRG PSEETGGAVFDHPAKIAKSTKSSSLNFSFPSLPTMGQMPGHSSD~~
~~TSGLSFSQPSCKTRVPHSKLDKGPTGANGHNTTQTIDYQDTV NMLHSLLS~~
~~AQGVQPTQPTAFEFVRPYS DYLNPRSGGISRSRGNTDKPRPPPLPSEPPP~~
~~PLPPLPK~~

Native sequence Amino acids M1 – K726 (end) of human Cyclin T1.
Residue M232 of the fusion protein is equivalent to M11 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*I sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGGAGGGAGAGAGGAAGAACAACAACAAACGGTGGTATTTCACTCGAGAACA
GCTGGAAAATAGCCCATCCCGTCGTTTTGGCGTGGACCCAGATAAAGAACTTTCTTATC
GCCAGCAGGCGGCCAATCTGCTTCAGGACATGGGGCAGCGTCTTAACGTCTCACAATTG
ACTATCAACACTGCTATAGTATACATGCATCGATTCTACATGATTCAAGTCCTTCACACA
GTTCCCTGGAAATTCTGTGGCTCCAGCAGCCTTGTTTTCTAGCAGCTAAAGTGGAGGAGC
AGCCCCAAAAATTGGAACATGTCATCAAGGTAGCACATACTTGTCTCCATCCTCAGGAA
TCCCTTCCTGATACTAGAAGTGAGGCTTATTTGCAACAAGTTCAAGATCTGGTCATTTT
AGAAAGCATAATTTTGCAGACTTTAGGCTTTGAACTAACAATTGACCACCCACATACTC
ATGTAGTAAAGTGCACCTCAACTTGTTTCGAGCAAGCAAGGACTTAGCACAGACTTCTTAC
TTCATGGCAACCAACAGCCTGCATTTGACCACATTTAGCCTGCAGTACACACCTCCTGT
GGTGGCCTGTGTCTGCATTACCTGGCTTGCAAGTGGTCCAATTGGGAGATCCCAGTCT
CAACTGACGGGAAGCACTGGTGGGAGTATGTTGACGCCACTGTGACCTTGGAACTTTTA
GATGAACTGACACATGAGTTTCTACAGATTTTGGAGAAAACCCCAACAGGCTCAAACG
CATTTGGAATTGGAGGGCATGCGAGGCTGCCAAGAAAACAAAAGCAGATGACCGAGGAA
CAGATGAAAAGACTTCAGAGCAGACAATCCTCAATATGATTTCCAGAGCTCTTCAGAC
ACAACCATTGCAGGTTTAATGAGCATGTCAACTTCTACCACAAGTGCAGTGCCTTCCCT
GCCAGTCTCCGAAGAGTCATCCAGCAACTTAACCAAGTGTGGAGATGTTGCCGGGCAAGC
GTTGGCTGTCCTCCCAACCTTCTTTCAAACCTAGAACCTACTCAGGGTCATCGGACTAGT
GAGAATTTAGCACTTACAGGAGTTGATCATTCTTACCACAGGATGGTTCAAATGCATT
TATTTCCAGAAAGCAGAATAGTAAGAGTGTGCCATCAGCTAAAGTGTCACTGAAAGAAT
ACCGCGCGAAGCATGCAGAAGAATTGGCTGCCCAGAAGAGGCAACTGGAGAACATGGAA
GCCAATGTGAAGTCACAATATGCATATGCTGCCCAGAATCTCCTTTCTCATCATGATAG
CCATTCTTCAGTCATTCTAAAAATGCCCATAGAGGGTTTCAGAAAACCCGGAGCGGCCTT
TTCTGGAAAAGGCTGACAAAACAGCTCTCAAAATGAGAATCCAGTGGCAGGTGGAGAT
AAAGCTGCGTCTTCAAAACCAGAGGAGATAAAAATGCGCATAAAAGTCCATGCTGCAGC
TGATAAGCACAATTCTGTAGAGGACAGTGTTACAAAGAGCCGAGAGCACAAAGAAAAGC
ACAAGACTCACCCATCTAATCATCATCATCATATAATCAACACTCACACAAGCACTCT
CATTCCCAACTTCCAGTTGGTACTGGGAACAAACGTCTTGGTGATCCAAAACATAGTAG
CCAGACAAGCAACTTAGCACATAAAACCTATAGCTTGTCTAGTTCTTTTTCTCTTCCA
GTTCTACTCGTAAAAGGGGACCCTCTGAAGAGACTGGAGGGGCTGTGTTTGATCATCCA
GCCAAGATTGCCAAGAGTACTAAATCCTCTTCCCTAAATTTCTCCTTCCCTTCACTTCC
TACAATGGGTGAGATGCCTGGGCATAGCTCAGACACAAGTGGCCTTTCTCTTTCACAGC
CCAGCTGTAAAACCTCGTGTCCCTCATTGAAACTGGATAAAGGGCCCACTGGGGCCAAT
GGTCACAACACGACCCAGACAATAGACTATCAAGACACTGTGAATATGCTTCACTCCCT
GCTCAGTGCCAGGGTGTTTCAGCCCACTCAGCCTACTGCATTTGAATTTGTTTCGTCTT
ATAGTGACTATCTGAATCCTCGGTCTGGTGGAATCTCCTCGAGATCTGGCAATACAGAC
AAACCCCGGCCACCACCTCTGCCATCAGAACCTCCTCCACCACCTTCCACCCCTTCTTAA
Gtaagcggccgc