

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Cyclin G1 [2 - 295]

Enzyme description:- Cyclin G1 [2 – 295]

Clone number:- DU 3018

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 60, 727.54 daltons

Average Mass 60, 766.91 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.34

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

Cyclin G1 [2 - 295]

<u>Protein</u>	Cyclin G1 [2 - 295]
<u>Clone number</u>	DU 3018
<u>Species</u>	Human
<u>Accession number</u>	NM_004060.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLOGWQATFGGGDHPKSDLEVLFGQPLGSEVLTTTDS QKLLHQNLALLEQESRCQPKVCGLRLIESAHDNGLRMTARLRDFEVKD LLSLTQFFGFDTEFSLAVNLLDRFLSKMKVQPKHLGCVGLSCFYLA KSIEEERNVPLATDLIRISQYRFTVSDLMRMEKIVLEKVCWKVKATTA FQFLQLYYSLLOENLPLERRNSINFERLEAQLKACHCRIIFSKAKPSV LALSIIALEIQAQKVELTEGIECLQKHSKINGRDLTFWQELVSKCLT EYSSNKCSKPNVQKLKWIVSGRTARQLKHSYYRITHLPTIPEMVP
<u>Native sequence</u>	Amino acids I2 – P295 (end) of human Cyclin G1. Residue I232 of fusion protein is equivalent to I2 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	Prescission site (LEVLFQGP) at residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGex6P-1

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Nucleotide sequence of insert

ggatccATAGAGGTACTGACAACAACTGACTCTCAGAACTGCTACAC
CAGCTGAATGCCCTGTTGGAACAGGAGTCTAGATGTCAGCCAAAGGTC
TGTGGTTTGAGACTAATTGAGTCTGCACACGATAATGGCCTCAGAATG
ACTGCAAGACTAAGGGACTTTGAAGTAAAAGATCTTCTTAGTCTAACT
CAGTTCTTTGGCTTTGACACAGAGACATTTTCTCTAGCTGTGAATTTA
CTGGACAGATTCCCTGTCTAAAATGAAGGTACAGCCCAAGCACCTTGGG
TGTGTTGGACTGAGCTGCTTTTATTTGGCTGTAAAATCAATAGAAGAG
GAAAGGAATGTCCCATTTGCAACTGACTTGATCCGAATAAGTCAATAT
AGGTTTACGGTTTCAGACTTGATGAGAATGGAAAAGATTGTATTGGAG
AAGGTGTGTTGGAAAGTCAAAGCTACTACTGCCTTTCAATTTCTGCAA
CTGTATTATTCACTCCTTCAAGAGAACTTGCCACTTGAAAGGAGAAAT
AGCATTAATTTTGAAGACTAGAAGCTCAACTGAAGGCATGTCATTGC
AGGATCATATTTTCTAAAGCAAAGCCTTCTGTGTTGGCATTGTCTATC
ATTGCATTAGAGATCCAAGCACAGAAGTGTGTAGAGTTAACAGAAGGA
ATAGAATGTCTTCAGAAACATTCCAAGATAAATGGCAGAGATCTGACC
TTCTGGCAAGAGCTTGTATCCAAATGTTTAACTGAATATTCATCAAAT
AAGTGTTCCAAACCAAATGTTTCAGAAGTTGAAATGGATTGTTTCTGGG
CGTACTGCACGGCAATTGAAGCATAGCTACTACAGAATAACTCACCTT
CCAACAATTCCTGAAATGGTCCCTtaagaattc