

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Cyclin Y [1 - 341]

Enzyme description:- Cyclin Y [1 - 341]

Clone number:- DU 35643

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 66, 118.58 daltons

Average Mass 66, 160.82 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.24

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

Division of Signal Transduction Therapy

Clone Data Sheet

Cyclin Y [1 - 341]

Protein Cyclin Y [1 - 341]

Clone number DU 35643

Species Human

Accession number NM_145012.5

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSMGNTTSCCVSSSPK
LRRNAHSRLESYRPDTDL SREDTGCNLQHISDRENIDDLNMEFNPSDHP
RASTIFLSKSQTDVREKRKSLFINHHPPGQIARKYSSCSTIFLDDSTVS
QPNLKYT IKCV ALAIYYHIKNRDPDGRMLLDIFDENLHPLSKSEVPPDY
DKHNPEQKQIYRFVRTLFSAAQLTAECAIVTLVYLERLLTYAEIDICPA
NWKRIVLGAILLASKVWDDQAVWNVDY CQILKDITVEDMNELE RQFLEL
LQFNINVPSSVYAKYYFDLRS LAEANNLSFPLEPLSRERAHKLEAISRL
CEDKYKDLRRSARKRSASADNLTLP RWSPAII S

Native sequence Amino acids M1 – S341 (end) of human Cyclin Y.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVL FQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P-1

Division of Signal Transduction Therapy

Nucleotide Sequence Of Insert

ggatccATGGGGAACACTACCTCGTGCTGCGTGTCGTCCAGTCCCAAGCT
CCGGAGGAATGCCCCTCCCGGCTGGAGTCTTACCGGCCAGACACGGACC
TGAGCCGCGAGGACACGGGCTGCAACCTGCAGCACATCAGCGACCGGGAG
AACATAGACGATTTGAACATGGAATTCAATCCTTCAGATCATCTCGGGC
CAGCACAATATTCCTCAGTAAATCTCAGACGGACGTGAGAGAAAAACGCA
AGAGTCTCTTCATTAACCATCATCCTCCAGGACAAATAGCAAGGAAATAC
AGTTCCTGCTCCACCATTTTCCTAGATGATAGCACAGTCAGTCAACCAA
CCTCAAGTATAACAATTAATGTGTCGCTCTTGCAATATATTATCACATCA
AAAACAGGGACCCAGATGGAAGGATGCTCTTAGATATTTTTGATGAAAAT
CTTCACCCCTCTTTGAAATCCGAAGTGCCACCAGATTATGACAAACACAA
CCCAGAGCAGAAGCAGATTTACCGGTTTCGTTCCGACACTGTTTCAGTGCTG
CTCAGCTGACGGCTGAATGTGCCATCGTCACCCTGGTGTACCTTGAAAGA
CTTTTAACATACGCAGAGATAGATATCTGTCCGGCCAACCTGGAAGCGGAT
TGTTTTAGGGGCGATCCTGCTGGCCTCCAAGGTGTGGGATGACCAGGCTG
TATGGAATGTGGATTACTGCCAGATCCTGAAAGACATCACGGTGGAGGAC
ATGAACGAGCTAGAGCGACAGTTTTCTTGAATTGCTGCAGTTCAACATCAA
TGTTCCCTCCAGTGTCTATGCCAAGTATTATTTTGATCTTCGTTCTCTGG
CAGAAGCGAACAACCTGAGCTTTCCCTTGGAGCCCCTGAGCAGGGAGAGG
GCTCACAAGCTTGAGGCCATCTCTCGCCTCTGCGAGGACAAGTACAAGGA
CCTAAGAAGATCCGCGAGGAAGCGCTCAGCCAGTGCAGACAACCTGACTC
TGCCCCGGTGGTCCCCAGCCATCATCTCTtaagcggccgc