Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Cyclin D1 [2 - 295]

Enzyme description:- Cyclin D1 [1 – 295]

Clone number:- DU 274

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 60, 442.53 daltons
Average Mass 60, 482.18 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.21

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 D1 [2 – 295]

Protein Cyclin D1 [2 – 295]

Clone number DU 274

Species Human

Accession number NM_053056.2

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**EHQLLCCEVETIRR**
AYPDANLLNDRVLRAMLKAEETCAPSVSYFKCVQKEVLPSMRKIVATWM
LEVCEEQKCEEEVFPLAMNYLDRFLSLEPVKKSRLQLLGATCMFVASKM
KETIPLTAEKLCIYTDNSIRPEELLQMELLLVNKLKWNLAAMTPHDFIE
HFLSKMPEAEENKQIIRKHAQTFVALCATDVKFISNPPSMVAAGSVVAA
VQGLNLRSPNNFLFYYRLTRFLSRVIKCDPDCLRACQEQIEALLESSLR
QAQQNMDPKAAEEEEEEEEEVDLACTPTDVRDVDI

Native sequence Amino acids E2 – I295 (end) of human Cyclin D1.
Residue E232 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

ggatccGAACACCAGCTCCTGTGCTGCGAAGTGGAAACCATCCGCCGCG
CGTACCCCGATGCCAACCTCCTCAACGACCGGGTGCTGCGGGCCATGCT
GAAGGCGGAGGAGACCTGCGCGCCCTCGGTGTCCTACTTCAAATGTGTG
CAGAAGGAGGTCCTGCCGTCCATGCGGAAGATCGTCGCCACCTGGATGC
TGGAGGTCTGCGAGGAACAGAAGTGCGAGGAGGAGGTCTTCCCGCTGGC
CATGAACTACCTGGACCGCTTCCTGTCGCTGGAGCCCGTGAAAAAGAGC
CGCCTGCAGCTGCTGGGGGCCACTTGCATGTTCGTGGCCTCTAAGATGA
AGGAGACCATCCCCCTGACGGCCGAGAAGCTGTGCATCTACACCGACAA
CTCCATCCGGCCCGAGGAGCTGCTGCAAATGGAGCTGCTCCTGGTGAAC
AAGCTCAAGTGGAACCTGGCCGCAATGACCCCGCACGATTTCATTGAAC
ACTTCCTCTCCAAAATGCCAGAGGCGGAGGAGAACAAACAGATCATCCG
CAAACACGCGCAGACCTTCGTTGCCCTCTGTGCCACAGATGTGAAGTTC
ATTTCCAATCCGCCCTCCATGGTGGCAGCGGGGAGCGTGGTGGCCGCAG
TGCAAGGCCTGAACCTGAGGAGCCCCAACAACTTCCTGTTCTACTACCG
CCTCACACGCTTCCTCTCCAGAGTGATCAAGTGTGACCCAGACTGCCTC
CGGGCCTGCCAGGAGCAGATCGAAGCCCTGCTGGAGTCAAGCCTGCGCC
AGGCCCAGCAGAACATGGACCCCAAGGCCGCCGAGGAGGAGGAAGAGGA
GGAGGAGGAGGTGGACCTGGCTTGCACACCCACCGACGTGCGGGACGTG
GACATCtgaggatcc