

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of Cyclin D3 [1 - 292]**

**Enzyme description:-** Cyclin D3 [1 – 292]

**Clone number:-** DU 1397

**Source:-** Recombinant

**Expression system:-** *E.coli*,

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 59, 305.18 daltons

Average Mass 59, 343.87 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.16

**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

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**Cyclin D3 [1 - 292]**

**Protein** Cyclin D3 [1 - 292]

**Clone number** DU 39025

**Species** Human

**Accession number** BC011616.2

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE  
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMDLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLOGWQATFGGGDHPKSDLEVLFOGPLGSMELLCCEGT  
**RHAPRAGPDPRLLDQORVLQSLRLLEERYVPRASYFQCVQREIKPHMR**  
**KMLAYWMLEVCEEQRCCEEVFPLAMNYLDRYLSQVPTRKAQLQLLGAV**  
**CMLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKWKDLA**  
**AVIAHDFLAFILHRLSLPRDRQALVKKHAQTFLALCATDYTFAMYPPS**  
**MIATGSI GAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEA**  
**ALRESLREASQTSSSPAPKAPRGSSSQGPSQTSTPTDVTAIHL**

**Native sequence** Amino acids M1 – L292 (end) of human Cyclin D3.  
Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

**Protease cleavage** Precission site (LEVLFOGP) at residues 221 – 228

**Cloning sites** *Bam*H1 and *Not*1 sites of pGex6P-1

*Division of Signal Transduction Therapy*

**Nucleotide**  
**sequence of insert**

ggatccATGGAGCTGCTGTGTTGCGAAGGCACCCGGCACGCGCCCCGG  
GCCGGCCGGACCCGCGGCTGCTGGGGGACCAGCGTGTCTGCAGAGC  
CTGCTCCGCCTGGAGGAGCGCTACGTACCCCGGCCTCCTACTTCCAG  
TGCGTGCAGCGGGAGATCAAGCCGCACATGCGGAAGATGCTGGCTTAC  
TGGATGCTGGAGGTATGTGAGGAGCAGCGCTGTGAGGAGGAAGTCTTC  
CCCCTGGCCATGAACTACCTGGATCGCTACCTGTCTTGCGTCCCCACC  
CGAAAGGCGCAGTTGCAGTCTCTGGGTGCGGTCTGCATGCTGCTGGCC  
TCCAAGCTGCGCGAGACCACGCCCTGACCATCGAAAACTGTGCATC  
TACACCGACCACGCTGTCTCTCCCCGCCAGTTGCGGGACTGGGAGGTG  
CTGGTCTTAGGGAAGCTCAAGTGGGACCTGGCTGCTGTGATTGCACAT  
GATTTCCTGGCCTTCATTCTGCACCGGCTCTCTTGCCCCGTGACCGA  
CAGGCCTTGGTCAAAAAGCATGCCAGACCTTTTTGGCCCTCTGTGCT  
ACAGATTATACCTTTGCCATGTACCCGCCATCCATGATCGCCACGGGC  
AGCATTGGGGCTGCAGTGCAAGGCCTGGGTGCCTGCTCCATGTCCGGG  
GATGAGCTCACAGAGCTGCTGGCAGGGATCACTGGCACTGAAGTGGAC  
TGCCTGCGGGCCTGTCAGGAGCAGATCGAAGCTGCACTCAGGGAGAGC  
CTCAGGGAAGCCTCTCAGACCAGCTCCAGCCAGCGCCCAAAGCCCC  
CGGGGCTCCAGCAGCCAAGGGCCAGCCAGACCAGCACTCCTACAGAT  
GTCACAGCCATACACCTGtaggcggccgc