

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of Cyclin A2 [171 – 432]**

**Enzyme description:-** Cyclin A2 [171 – 432]

**Clone number:-** DU 1064

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 56, 806.14 daltons

Average Mass 56, 842.92 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.96

**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 A2 [171 – 432]**

<b><u>Protein</u></b>	Cyclin A2 [171 - 432]
<b><u>Clone Number</u></b>	DU 1064
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_001237
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEELHYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIIPQIDKY LKSSKYIAWPLQGQWQATFGGGDHPKSDLEVLFGQPLGS <b>SVNEVPDYH</b> <b>EDIHTYLREMEVKCKPKVGYMKKQPDITNSMRAILVDWLVEVGEEYKL</b> <b>QNETLHLAVNYIDRFLSSMSVLRGKLQLVGTAAMLLASKFEEIYPPEV</b> <b>AEFVYITDDTYTKQVLRMEHLVLRKVLTFDLAAPTQVNFQFLTQYFLHQQ</b> <b>PANCKVESLAMFLGELSLIDADPYLKYLPQVIAAGAFHLALYTVTGQS</b> <b>WPESLIRKTGYTLESCLKPCLMDLHQTYLKAPQHAQQSIREKYKNSKYH</b> <b>GVSLLNPPETLNL</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids S171 – L432 (end) of human Cyclin A2. Residue S232 of the fusion protein is S171 of the native protein. The GST tag is located between residues 1 - 220.</p>
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFQGPL</u> ) at residues 221 – 229
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Eco</i> R1 site of pGEX6P-1

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**Nucleotide  
sequence of  
insert**

ggatccAGTGTTAATGAAGTACCAGACTACCATGAGGATATTCACACA  
TACCTTAGGGAAATGGAGGTTAAATGTAAACCTAAAGTGGGTACATG  
AAGAAACAGCCAGACATCACTAACAGTATGAGAGCTATCCTCGTGGAC  
TGGTTAGTTGAAGTAGGAGAAGAATATAAACTACAGAATGAGACCCTG  
CATTTGGCTGTGAACTACATTGATAGGTTCCCTGTCTTCCATGTCAGTG  
CTGAGAGGAAAACCTCAGCTTGTGGGCACTGCTGCTATGCTGTTAGCC  
TCAAAGTTTGAAGAAATATACCCCCAGAAGTAGCAGAGTTTGTGTAC  
ATTACAGATGATACCTACACCAAGAAACAAGTTCTGAGAATGGAGCAT  
CTAGTTTTGAAAGTCCTTACTTTTGACTTAGCTGCTCCAACAGTAAAT  
CAGTTTCTTACCCAATACTTTCTGCATCAGCAGCCTGCAAACCTGCAAA  
GTTGAAAGTTTAGCAATGTTTTTGGGAGAATTAAGTTTGATAGATGCT  
GACCATACCTCAAGTATTTGCCATCAGTTATTGCTGGAGCTGCCTTT  
CATTTAGCACTCTACACAGTCACGGGACAAAGCTGGCCTGAATCATTA  
ATACGAAAGACTGGATATACCCTGGAAAGTCTTAAGCCTTGTCTCATG  
GACCTTCACCAGACCTACCTCAAAGCACACAGCATGCACAACAGTCA  
ATAAGAGAAAAGTACAAAATTCAAAGTATCATGGTGTCTCTCCTC  
AACCCACCAGAGACACTAAATCTGtaagaattc