Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active CDK2 [2 – 298] / Cyclin A2 [171 – 432]

Enzyme description:- CDK2 [2 – 298] / Cyclin A2 [171 – 432]

Clone number:-

CDK2 [2 – 298]	DU 1043
Cyclin A2 [171 – 432]	DU 1064

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST for both proteins

Purification method:-
Following expression separately, bacterial pellets are mixed prior to lysis, purified by GSH Sepharose chromatography and the GST tags cleaved with PreScission protease. The cleaved proteins are purified by gel filtration chromatography.

Calculated molecular mass:-

Monoisotopic	60, 583.62 daltons (CDK2 after cleaving)
	29, 999.50 (Cyclin A after cleaving)
Average Mass	33, 942.47 daltons (CDK2 after cleaving)
	30, 018.76 (Cyclin A after cleaving)

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8,81 CDK2 cleaved and 6.14 Cyclin A cleaved

Purity:- 90 %

Activation protocol:-
CDK2 / Cyclin A2 (1 μM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM MgAc, 0.1 mM ATP with 1 μM active CAK1 [DU 1089] at 30 °C for 30 min. Following activation, the CAK1 is removed from the preparation by GSH Sepharose chromatography.

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 0.1 mM sodium vanadate, 10 mM magnesium acetate

Division of Signal Transduction Therapy

Substrate:- Histone H1 Final concentration: 1 mg/ml

Clone Data Sheet

CDK2 [2 – 298]

Protein	CDK2 [2 – 298]
Clone number	DU 1043
Species	Human
Accession number	NM_001798
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**ENFQKVEKI GEGTYGVVYKARNKLTGEVVALKKIRLDTETEGVPSTAIREISLLKEL NHPNIVKLLDVIHTENKLYLVFEFLHQDLKKFMDASALTGIPLPLIKS YLFQLLQGLAFCHSHRVLHRDLKPQNLLINTEGAIKLADFGLARAFGV PVRTYTHEVVTLWYRAPEILLGCKYYSTAVDIWSLGCIFAEMVTRRAL FPGDSEIDQLFRIFRTLGTPDEVVWPGVTSMPDYKPSFPKWARQDFSK VVPPLDEDGRSLLSQMLHYDPNKRISAKAALAHPFFQDVTKPVPHLRL**
Native sequence	Amino acids E2 – L298 (end) of human CDK2. Residue E232 of the fusion protein is equilivalent to E2 of the native enzyme. The GST tag is located between residues 1- 220.
Protease cleavage	PreScission (LEVLFQGP) at residues 221 – 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCGAGAACTTCCAAAAGGTGGAAAAGATCGGAGAGGGCACGTAC
GGAGTTGTGTACAAAGCCAGAAACAAGTTGACGGGAGAGGTGGTGGCG
CTTAAGAAAATCCGCCTGGACACTGAGACTGAGGGTGTGCCCAGTACT
GCCATCCGAGAGATCTCTCTGCTTAAGGAGCTTAACCATCCTAATATT
GTCAAGCTGCTGGATGTCATTCACACAGAAAATAAACTCTACCTGGTT
TTTGAATTTCTGCACCAAGATCTCAAGAAATTCATGGATGCCTCTGCT
CTCACTGGCATTCCTCTTCCCCTCATCAAGAGCTATCTGTTCCAGCTG
CTCCAGGGCCTAGCTTTCTGCCATTCTCATCGGGTCCTCCACCGAGAC
CTTAAACCTCAGAATCTGCTTATTAACACAGAGGGGGCCATCAAGCTA
GCAGACTTTGGACTAGCCAGAGCTTTTGGAGTCCCTGTTCGTACTTAC
ACCCATGAGGTGGTGACCCTGTGGTACCGAGCTCCTGAAATCCTCCTG
GGCTGCAAATATTATTCCACAGCTGTGGACATCTGGAGCCTGGGCTGC
ATCTTTGCTGAGATGGTGACTCGCCGGGCCCTATTCCCTGGAGATTCT
GAGATTGACCAGCTCTTCCGGATCTTTCGGACTCTGGGGACCCCAGAT
GAGGTGGTGTGGCCAGGAGTTACTTCTATGCCTGATTACAAGCCAAGT
TTCCCCAAGTGGGCCCGGCAAGATTTTAGTAAAGTTGTACCTCCCCTG
GATGAAGATGGACGGAGCTTGTTATCGCAAATGCTGCACTACGACCCT
AACAAGCGGATTTCGGCCAAGGCAGCCCTGGCTCACCCTTTCTTCCAG
GATGTGACCAAGCCAGTACCCCATCTTCGACTCtgagaattc

Division of Signal Transduction Therapy

CLONE DATA SHEET

Cyclin A2 [171 – 432]

Protein	Cyclin A2 [171 - 432]
Clone Number	DU 1064
Species	Human
Accession number	NM_001237
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SVNEVPDYH** **EDIHTYLREMEVKCKPKVGYMKKQPDITNSMRAILVDWLVEVGEEYKL** **QNETLHLAVNYIDRFLSSMSVLRGKLQLVGTAAMLLASKFEEIYPPEV** **AEFVYITDDTYTKKQVLRMEHLVLKVLTFDLAAPTVNQFLTQYFLHQQ** **PANCKVESLAMFLGELSLIDADPYLKYLPSVIAGAAFHLALYTVTGQS** **WPESLIRKTGYTLESLKPCLMDLHQTYLKAPQHAQQSIREKYKNSKYH** **GVSLLNPPETLNL**
Native sequence	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.
Protease cleavage	PreScission (LEVLFQGPL) at residues 221 – 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCAGTGTTAATGAAGTACCAGACTACCATGAGGATATTCACACA
TACCTTAGGGAAATGGAGGTTAAATGTAAACCTAAAGTGGGTTACATG
AAGAAACAGCCAGACATCACTAACAGTATGAGAGCTATCCTCGTGGAC
TGGTTAGTTGAAGTAGGAGAAGAATATAAACTACAGAATGAGACCCTG
CATTTGGCTGTGAACTACATTGATAGGTTCCTGTCTTCCATGTCAGTG
CTGAGAGGAAAACTTCAGCTTGTGGGCACTGCTGCTATGCTGTTAGCC
TCAAAGTTTGAAGAAATATACCCCCCAGAAGTAGCAGAGTTTGTGTAC
ATTACAGATGATACCTACACCAAGAAACAAGTTCTGAGAATGGAGCAT
CTAGTTTTGAAAGTCCTTACTTTTGACTTAGCTGCTCCAACAGTAAAT
CAGTTTCTTACCCAATACTTTCTGCATCAGCAGCCTGCAAACTGCAAA
GTTGAAAGTTTAGCAATGTTTTTGGGAGAATTAAGTTTGATAGATGCT
GACCCATACCTCAAGTATTTGCCATCAGTTATTGCTGGAGCTGCCTTT
CATTTAGCACTCTACACAGTCACGGGACAAAGCTGGCCTGAATCATTA
ATACGAAAGACTGGATATACCCTGGAAAGTCTTAAGCCTTGTCTCATG
GACCTTCACCAGACCTACCTCAAAGCACCACAGCATGCACAACAGTCA
ATAAGAGAAAAGTACAAAAATTCAAAGTATCATGGTGTTTCTCTCCTC
AACCCACCAGAGACACTAAATCTGtaagaattc