Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CDK2 [1 – 298] / Cyclin A [171 – 432]

Enzyme description:-	CDK2 [1 – 298] / Cyclin A [171 – 432]
Clone number:-	DU 43557
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal GST for Cyclin A (GST tag cleaved) CDK2 untagged
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	33, 908.02 daltons [CDK2],	30, 410.71 daltons [Cyclin A cleaved]
Average Mass	33, 929.53 daltons [CDK2],	30, 430.22 daltons [Cyclin A cleaved]

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	8.80 [CDK2] and 6.16 [Cyclin A cleaved]
Purity:-	>80 %
Activation protocol:-	Activated by GST-CAK1 [DU 1089]

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

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

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

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Clone Data Sheet

CDK2 [1 – 298]

Protein	CDK2 [1 – 298]
Clone number	DU 43557
Species	Human
Accession number	NM_001798.4
Tags	N-terminal GST
Bacterially expressed protein	**MENFQKVEKIGEGTYGVVYKARNKLTGEVVALKKIRLDTETEGVPSTAIREISLLKELNHPNIVKLLDVIHTENKLYLVFEFLHQDLKKFMDASALTGIPLPLIKSYLFQLLQGLAFCHSHRVLHRDLKPQNLLINTEGAIKLADFGLARAFGVPVRTYTHEVVTLWYRAPEILLGCKYYSTAVDIWSLGCIFAEMVTRRALFPGDSEIDQLFRIFRTLGTPDEVVWPGVTSMPDYKPSFPKWARQDFSKVVPPLDEDGRSLLSQMLHYDPNKRISAKAALAHPFFQDVTKPVPHLRL**
Native sequence	Amino acids M1 – L298 (end) of human CDK2.
Cloning sites	*Eco*R1 and *Not*1 site of pGEX6P-1

Nucleotide sequence of insert

ATGGAGAACTTCCAAAAGGTGGAAAAGATCGGAGAGGGCACGTACGGA
GTTGTGTACAAAGCCAGAAACAAGTTGACGGGAGAGGTGGTGGCGCTT
AAGAAAATCCGCCTGGACACTGAGACTGAGGGTGTGCCCAGTACTGCC
ATCCGAGAGATCTCTCTGCTTAAGGAGCTTAACCATCCTAATATTGTC
AAGCTGCTGGATGTCATTCACACAGAAAATAAACTCTACCTGGTTTTT
GAATTTCTGCACCAAGATCTCAAGAAATTCATGGATGCCTCTGCTCTC
ACTGGCATTCCTCTTCCCCTCATCAAGAGCTATCTGTTCCAGCTGCTC
CAGGGCCTAGCTTTCTGCCATTCTCATCGGGTCCTCCACCGAGACCTT
AAACCTCAGAATCTGCTTATTAACACAGAGGGGGCCATCAAGCTAGCA
GACTTTGGACTAGCCAGAGCTTTTGGAGTCCCTGTTCGTACTTACACC
CATGAGGTGGTGACCCTGTGGTACCGAGCTCCTGAAATCCTCCTGGGC
TGCAAATATTATTCCACAGCTGTGGACATCTGGAGCCTGGGCTGCATC
TTTGCTGAGATGGTGACTCGCCGGGCCCTATTCCCTGGAGATTCTGAG
ATTGACCAGCTCTTCCGGATCTTTCGGACTCTGGGGACCCCAGATGAG
GTGGTGTGGCCAGGAGTTACTTCTATGCCTGATTACAAGCCAAGTTTC
CCCAAGTGGGCCCGGCAAGATTTTAGTAAAGTTGTACCTCCCCTGGAT
GAAGATGGACGGAGCTTGTTATCGCAAATGCTGCACTACGACCCTAAC
AAGCGGATTTCGGCCAAGGCAGCCCTGGCTCACCCTTTCTTCCAGGAT
GTGACCAAGCCAGTACCCCATCTTCGACTCtga

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CLONE DATA SHEET

Cyclin A [171 – 432]

Protein	Cyclin A [171 - 432]
Clone Number	DU 43557
Species	Human
Accession number	NM_001237.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SVNEVPDYHEDIHTYLREMEVKCKPKVGYMKKQPDITNSMRAILVDWLVEVGEEYKLQNETLHLAVNYIDRFLSSMSVLRGKLQLVGTAAMLLASKFEEIYPPEVAEFVYITDDTYTKKQVLRMEHLVLKVLTFDLAAPTVNQFLTQYFLHQQPANCKVESLAMFLGELSLIDADPYLKYLPSVIAGAAFHLALYTVTGQSWPESLIRKTGYTLESLKPCLMDLHQTYLKAPQHAQQSIREKYKNSKYHGVSLLNPPETLNL**
Native sequence	Amino acids S171 – L432 (end) of human Cyclin A. Residue S232 of the fusion protein is S171 of the native protein. The GST tag is located between residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGP) at residues 221 – 228
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

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ggatccAGTGTTAATGAAGTACCAGACTACCATGAGGATATTCACACA
TACCTTAGGGAAATGGAGGTTAAATGTAAACCTAAAGTGGGTTACATG
AAGAAACAGCCAGACATCACTAACAGTATGAGAGCTATCCTCGTGGAC
TGGTTAGTTGAAGTAGGAGAAGAATATAAACTACAGAATGAGACCCTG
CATTTGGCTGTGAACTACATTGATAGGTTCCTGTCTTCCATGTCAGTG
CTGAGAGGAAAACTTCAGCTTGTGGGCACTGCTGCTATGCTGTTAGCC
TCAAAGTTTGAAGAAATATACCCCCCAGAAGTAGCAGAGTTTGTGTAC
ATTACAGATGATACCTACACCAAGAAACAAGTTCTGAGAATGGAGCAT
CTAGTTTTGAAAGTCCTTACTTTTGACTTAGCTGCTCCAACAGTAAAT
CAGTTTCTTACCCAATACTTTCTGCATCAGCAGCCTGCAAACTGCAAA
GTTGAAAGTTTAGCAATGTTTTTGGGAGAATTAAGTTTGATAGATGCT
GACCCATACCTCAAGTATTTGCCATCAGTTATTGCTGGAGCTGCCTTT
CATTTAGCACTCTACACAGTCACGGGACAAAGCTGGCCTGAATCATTA
ATACGAAAGACTGGATATACCCTGGAAAGTCTTAAGCCTTGTCTCATG
GACCTTCACCAGACCTACCTCAAAGCACCACAGCATGCACAACAGTCA
ATAAGAGAAAAGTACAAAAATTCAAAGTATCATGGTGTTTCTCTCCTC
AACCCACCAGAGACACTAAATCTGTAAGAATTCAATAATTTTGTTTAA
CTTTAAGAAGGAGATATACATATGGAGAACTTCCAAAAGGTGGAAAAG
ATCGGAGAGGGCACGTACGGAGTTGTGTACAAAGCCAGAAACAAGTTG
ACGGGAGAGGTGGTGGCGCTTAAGAAAATCCGCCTGGACACTGAGACT
GAGGGTGTGCCCAGTACTGCCATCCGAGAGATCTCTCTGCTTAAGGAG
CTTAACCATCCTAATATTGTCAAGCTGCTGGATGTCATTCACACAGAA
AATAAACTCTACCTGGTTTTTGAATTTCTGCACCAAGATCTCAAGAAA
TTCATGGATGCCTCTGCTCTCACTGGCATTCCTCTTCCCCTCATCAAG
AGCTATCTGTTCCAGCTGCTCCAGGGCCTAGCTTTCTGCCATTCTCAT
CGGGTCCTCCACCGAGACCTTAAACCTCAGAATCTGCTTATTAACACA
GAGGGGGCCATCAAGCTAGCAGACTTTGGACTAGCCAGAGCTTTTGGA
GTCCCTGTTCGTACTTACACCCATGAGGTGGTGACCCTGTGGTACCGA
GCTCCTGAAATCCTCCTGGGCTGCAAATATTATTCCACAGCTGTGGAC
ATCTGGAGCCTGGGCTGCATCTTTGCTGAGATGGTGACTCGCCGGGCC
CTATTCCCTGGAGATTCTGAGATTGACCAGCTCTTCCGGATCTTTCGG
ACTCTGGGGACCCCAGATGAGGTGGTGTGGCCAGGAGTTACTTCTATG
CCTGATTACAAGCCAAGTTTCCCCAAGTGGGCCCGGCAAGATTTTAGT
AAAGTTGTACCTCCCCTGGATGAAGATGGACGGAGCTTGTTATCGCAA
ATGCTGCACTACGACCCTAACAAGCGGATTTCGGCCAAGGCAGCCCTG
GCTCACCCTTTCTTCCAGGATGTGACCAAGCCAGTACCCCATCTTCGA
CTCtgagcggccgc
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