

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active CDK7 [1 – 346] + MAT1 [2 – 309] + Cyclin H [1 - 323]

Enzyme description:- CDK7 [1 - 346] + MAT1 [2 – 309] + Cyclin H [1 – 323]

Clone number:- DU 49574

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- CDK7 C-terminal His(6) tag
MAT1 N-terminal GST tag
Cyclin H (untagged)

Purification method:- Ni²⁺-NTA agarose

Calculated molecular mass for CDK7:-

Monoisotopic 39, 949.92 daltons
Average Mass 39, 975.35 daltons
[cysteines reduced, methionines have not been oxidised]

Calculated molecular mass for MAT1:-

Monoisotopic 62, 475.90 daltons
Average Mass 62, 515.93 daltons
[cysteines reduced, methionines have not been oxidised]

Calculated molecular mass for Cyclin H:-

Monoisotopic 37, 619.20 daltons
Average Mass 37, 643.45 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.67 for CDK7
5.77 for MAT1
6.73 for Cyclin H

Purity:- >80 %

Activation protocol:- Constitutively active

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Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

YSPTSPSYSPTSPSYSPSPTSPSKKK

Final concentration: 300 uM

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Clone Data Sheet CDK7 [1 - 346]

Protein CDK7 [1 - 346]

Clone number DU 49574

Species Human

Accession number NM_001799.3

Tags C-terminal His6

Baculovirus CDK7 expressed protein MALDVKSRAKRYEKLDLFLGEGQFATVYKARDKNTNQIIVAIAKKIKL
GHRSEAKDGINRTALREIKLLQELSHPNIIIGLLDAFGHKSNIISLV
FDFMETDLEVI IKDNSLVLTSPHIKAYMLMTLQGLEYLHQHWILH
RDLKPNNLLLDENGVKLADFGGLAKSFGSPNRAYTHQVVTRWYRA
PELLFGARMYGVGVDMWAVGCILAELLRVPFLPGDSDLQDLTRI
FETLGTPTEEQWPDMSLPDYVTFKSFPGIPLHHIFSAAGDILLD
LIQGLFLFNPCARITATQALKMKYFSNRPGPTPGCQLPRPNCPE
TLKEQSNPALAIKRRKTEALEQGGLPKKLIFGGHHHHHH

Native sequence Amino acids M1 – F346 (end) of human CDK7.
Residue M1 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 349 - 354.

Protease cleavage None

Cloning sites *Bam*H1 and *Not*I sites of pFastBac DM.

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Nucleotide sequence of insert

ggatccgaaaccATGGCTCTGGACGTGAAGTCTCGGGCAAAGCGT
TATGAGAAGCTGGACTTCCTTGGGGAGGGACAGTTTGCCACCGTT
TACAAGGCCAGAGATAAGAACACCAACCAATTGTCGCCATTAAG
AAAATCAAACCTTGGACATAGATCAGAAGCTAAAGATGGTATAAAT
AGAACCGCCTTAAGAGAGATAAAAATTATTACAGGAGCTAAGTCAT
CCAAATATAATTGGTCTCCTTGATGCTTTTGGACATAAATCTAAT
ATTAGCCTTGTCTTTGATTTTATGGAACTGATCTAGAGGTTATA
ATAAAGGATAATAGTCTTGTGCTGACACCATCACACATCAAAGCC
TACATGTTGATGACTCTTCAAGGATTAGAATATTTACATCAACAT
TGGATTCTACATAGGGATCTGAAACCAAACAACCTTGTTGCTAGAT
GAAAATGGAGTTCTAAAACCTGGCAGATTTTGGCCTGGCCAAATCT
TTTGGGAGCCCCAATAGAGCTTATACACATCAGGTTGTAACCAGG
TGGTATCGGGCCCCGAGTTACTATTTGGAGCTAGGATGTATGGT
GTAGGTGTGGACATGTGGGCTGTTGGCTGTATATTAGCAGAGTTA
CTTCTAAGGGTTCCTTTTTTGGCCAGGAGATTCAGACCTTGATCAG
CTAACAAGAATATTTGAACTTTGGGCACACCAACTGAGGAACAG
TGGCCGGACATGTGTAGTCTTCCAGATTATGTGACATTTAAGAGT
TTCCCTGGAATACCTTTGCATCACATCTTCAGTGCAGCAGGAGAC
GACTTACTAGATCTCATAACAAGGCTTATTCTTATTTAATCCATGT
GCTCGAATTACGGCCACACAGGCACTGAAAATGAAGTATTTTCAGT
AATCGGCCAGGGCCAACACCTGGATGTCAGCTGCCAAGACCAAAC
TGTCCAGTGGAAACCTTAAAGGAGCAATCAAATCCAGCTTTGGCA
ATAAAAAGGAAAAGAACAGAGGCCTTAGAACAAGGAGGATTGCC
AAGAACTAATTTTTTGGCGGACATCACCATCACCATCACtaagcg
gccgc

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Clone Data Sheet MAT1 [2 - 309]

Protein MAT1 [2 - 309]

Clone number DU 49574

Species Human

Accession number NM_002431.3

Tags N-terminal GST

Baculovirus MAT1 expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVLV
QGPLGSDDQGCP **RCKTTKYRNP** **SLKLMVNVCGHTL** **CESCVDLLFV**
RGAGNCP **ECGTPLRKS** **NFRVQLF** **EDPTVDKEVE** **IRKKVLKIYNKR**
EEDFPSLREY **NDFLEEV** **EIVFNLTNNVDL** **DNTKKKMEIYQ** **KENK**
DVIQKNKLKLTRE **QEELEEA** **LEVERQENEQRRLFIQKEEQ** **LOQIL**
KRKNKQAF **LDELESSDLP** **VALLLAQH** **KDRSTQLEM** **QLEKPKPVKP**
VTFSTGIKMGQ **HISLAPIHKLEEA** **LYEYQPLQIETYGPHVPELEM**
LGRLGYLNHVRAAS **PDLAGGYTSS** **LACHRALQDAF** **SGLFWQPS**

Native sequence Amino acids D2 – S309 (end) of human MAT1.
Residue D232 of the fusion protein is equivalent to D2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission site (LEVLFQGP) residues 221 – 228

Cloning sites *EcoR1* and *Not1* sites of pFastBac DM.

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**Nucleotide
sequence of insert**

gaattcgaaacccATGTCCCCTATACTAGGTTATTGGAAAATTAAG
GGCCTTGTGCAACCCACTCGACTTCTTTTGGAAATATCTTGAAGAA
AAATATGAAGAGCATTGTATGAGCGCGATGAAGGTGATAAATGG
CGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCT
TATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATC
ATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCA
AAAGAGCGTGCAAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGAT
ATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAA
ACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAA
ATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGAT
CATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTT
GTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTA
GTTTGTTTTAAAAACGTATTGAAGCTATCCCACAAATTGATAAG
TACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGG
CAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTG
GAAGTTCTGTTCCAGGGCCCCCTGGGATCCGACGATCAGGGTTGC
CCTCGGTGTAAGACCACCAATATCGGAACCCCTCCTTGAAGCTG
ATGGTGAATGTGTGCGGACACACTCTCTGTGAAAGTTGTGTAGAT
TTACTGTTTGTGAGAGGAGCTGGAAACTGCCCTGAGTGTGGTACT
CCACTCAGAAAGAGCAACTTCAGGGTACAACCTCTTTGAAGATCCC
ACTGTTGACAAGGAGGTTGAGATCAGGAAAAAAGTGCTAAAGATA
TACAATAAAAGGGAAGAAGATTTTCCTAGTCTAAGAGAATACAAT
GATTTCTTGGAAAGAAGTGAAGAAATTGTTTTCAACTTGACCAAC
AATGTGGATTTGGACAACACCAAAAAGAAAATGGAGATATACCAA
AAGGAAAACAAAGATGTTATTCAGAAAAATAAATTAAGCTGACT
CGAGAACAGGAAGAAGTGAAGAAGCTTTAGAAGTGAACGACAG
GAAAATGAACAAAGAAGATTATTTATACAAAAAGAACAACCTG
CAGCAGATTCTAAAAAGGAAGAATAAGCAGGCTTTTTTTAGATGAG
CTGGAGAGTTCTGATCTCCCTGTTGCTCTGCTTTTTGGCTCAGCAT
AAAGATAGATCTACCCAATTAGAAATGCAACTTGAGAAACCCAAA
CCTGTAAAACCAGTGACGTTTTTCCACAGGCATCAAAATGGGTCAA
CATATTTCACTGGCACCTATTCACAAGCTTGAAGAAGCTCTGTAT
GAATACCAGCCACTGCAGATAGAGACATATGGACCACATGTTCCCT
GAGCTTGAGATGCTAGGAAGACTTGGGTATTTAAACCATGTCAGA
GCTGCCTCACCACAGGACCTTGCTGGAGGCTATACTTCTTCTCTT
GCTTGTACAGAGCACTACAGGATGCATTCAGTGGGCTTTTTCTGG
CAGCCCAGTtaagcggccgc

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Clone Data Sheet Cyclin H [1 - 323]

Protein Cyclin H [1 - 323]

Clone number DU 49574

Species Human

Accession number NM_001239.2

Tags Untagged

Baculovirus Cyclin H expressed protein **MYHNSSQKRHWTFSSSEQLARLRADANRKFRCCKAVANGKVLPNDFVFLPHEEMTLCKYYEKRLLEFCSVFKPAMPRSVVGTACMYFKRFYLNNSVMEYHPRIIMLTCAFLACKVDEFNVSSPQFVGNLRESPLGQEKALEQILEYELLLIQQLNFHLIVHNPYRPFEGFLIDLKTRYPILENPEILRKTADDFLNRIALTDAYLLYTPSQIALTAILSSASRAGITMESYLSESLMLKENRTCLSQLLDIMKSMRNLVKKYEP RSEEVAVLKQKLERCHSAELALNVITKKRKG YEDDDYVSKKSKHEEEEWTDL VESL**

Native sequence Amino acids M1 – L323 (end) of human Cyclin H. Residue M1 of the fusion protein is equivalent to M1 of the native enzyme.

Protease cleavage None available

Cloning sites *Spe*1 and *Nru*1 sites of pFastBac DM.

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**Nucleotide
sequence of insert**

ATGTACCACAACAGTAGTCAGAAGCGGCACTGGACCTTCTCCAGC
GAGGAGCAGCTGGCAAGACTGCGGGCTGACGCCAACCGCAAATTC
AGATGCAAAGCCGTGGCCAACGGGAAGGTTCTTCCGAATGATCCA
GTCCTTTCTTGAGCCTCATGAAGAAATGACACTCTGCAAATACTAT
GAGAAAAGGTTATTGGAATTCTGTTCCGGTGTTTAAGCCAGCAATG
CCAAGATCTGTTGTGGGTACGGCTTGTATGTATTTCAAACGTTTT
TATCTTAATAACTCAGTAATGGAATATCACCCAGGATAATAATG
CTCACTTGTGCATTTTTGGCCTGCAAAGTAGATGAATTC AATGTA
TCTAGTCCTCAGTTTGTGGAAACCTCCGGGAGAGTCTCTTGGA
CAGGAGAAGGCACTTGAACAGATACTGGAATATGAACTACTTCTT
ATACAGCAACTTAATTTCCACCTTATTGTCCACAATCCTTACAGA
CCATTTGAGGGCTTCCTCATCGACTTAAAGACCCGCTATCCCATA
TTGGAGAATCCAGAGATTTTGAGGAAAACAGCTGATGACTTTCTT
AATAGAATTGCATTGACGGATGCTTACCTTTTATACACACCTTCC
CAAATTGCCCTGACTGCCATTTTATCTAGTGCCTCCAGGGCTGGA
ATTACTATGGAAAGTTATTTATCAGAGAGTCTGATGCTGAAAGAG
AACAGAACTTGCCTGTCACAGTTACTAGATATAATGAAAAGCATG
AGAACTTAGTAAAGAAGTATGAACCACCCAGATCTGAAGAAGTT
GCTGTTCTGAAACAGAAGTTGGAGCGATGTCATTCTGCTGAGCTT
GCACTTAACGTAATCACGAAGAAGAGGAAAGGCTATGAAGATGAT
GATTACGTCTCAAAGAAATCCAAACATGAGGAGGAAGAATGGACT
GATGACGACCTGGTAGAATCTCTCt aa