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Certificate of Analysis

Cdk7/cyclinH/MAT1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-476, 14-476-K, 14-476M
Parent Lot # 1989530

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Complex of *C*-terminal 6His-tagged human full length cdk7, full length untagged cyclin H and *N*-terminal GST-tagged human full length MAT1. These are expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Combined purity 77.4% by SDS-PAGE and Coomassie blue staining. cdk7 MW = 39.9kDa, cyclinH MW = 37.8kDa, MAT1 MW = 62.8kDa.

Specific Activity (Parent lot# 1989530): 25U/mg, where one unit of cdk7/cyclinH/MAT1 activity is defined as 1nmol phosphate incorporated into 500µM ((YSPTSPS)3.KKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.712mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

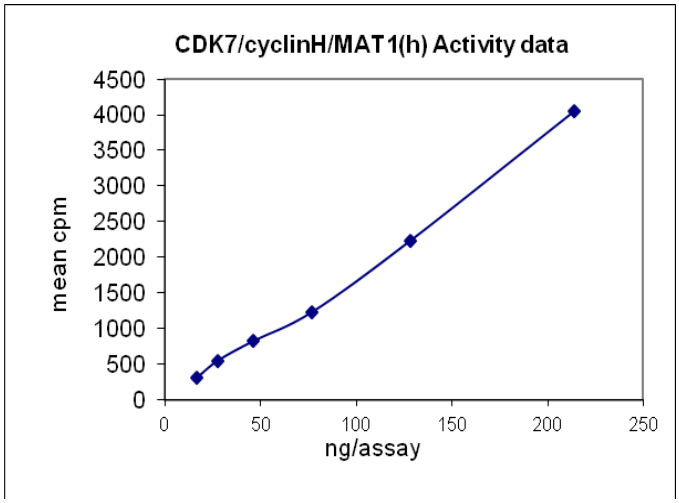
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

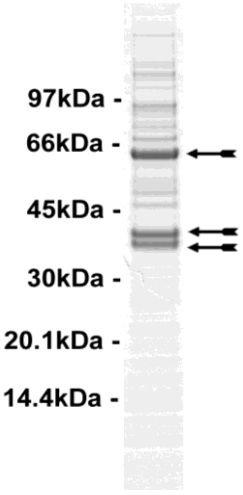
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 17–214ng of this lot of enzyme phosphorylated 500µM ((YSPTSPS)3.KKKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as cdk7/cyclinH/MAT1 with the translated native cyclinH sequence listed on page four and the translated native MAT1 sequence listed on page five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of cdk7/cyclinH/MAT1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **((YSPTSPS)3.KKKK):** Use at a final assay concentration of 500µM. Prepare a 5mM stock and add 2.5µl of stock per assay point.
3. **cdk7/cyclinH/MAT1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 17–214ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of (**(YSPTSPS) 3.KKKK)**.
3. Add **2.5µl (17–214ng) cdk7/cyclinH/MAT1, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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cdk7 Sequence Information

Protein Human cdk7

Accession number GenBank X79193

Tags *C*-terminal 6His

Native sequence M1 of native protein is equivalent to M1 of recombinant protein

Recombinant cdk7 amino acid sequence:

```
   1 MALDVKSRAK RYEKLDFLGE GQFATVYKAR DKNTNQIVAI KKIKLGHRSE AKDGINRTAL
  61 REIKLLQELS HPNIIGLLDA FGHKSNISLV FDFMETDLEV IIKDNSLVLT PSHIKAYMLM
 121 TLQGLEYLHQ HWILHRDLKP NNLLLDENGV LKLADFGLAK SFGSPNRAYT HQVVTRWYRA
 181 PELLFGARMY GVGVDMWAVG CILAELLLRV PFLPGDSDLD QLTRIFETLG TPTEEQWPDM
 241 CSLPDYVTFK SFPGIPLHHI FSAAGDDLLD LIQGLFLFNP CARITATQAL KMKYFSNRPG
 301 PTPGCQLPRP NCPVETLKEQ SNPALAIKRK RTEALEQGGL PKKLIFHHHH HH
```

Recombinant cdk7 nucleotide sequence:

```
   1 atggctctgg acgtgaagtc tcgggcaaag cgttatgaga agctggactt ccttggggag
  61 ggacagtttg ccaccgttta caaggccaga gataagaata ccaaccaaat tgtcgccatt
 121 aagaaaatca aacttggaca tagatcagaa gctaaagatg gtataaatag aaccgcctta
 181 agagagataa aattattaca ggagctaagt catccaaata taattggtct ccttgatgct
 241 tttggacata aatctaatat tagccttgtc tttgatttta tggaaactga tctagaggtt
 301 ataataaagg ataatagtct tgtgctgaca ccatcacaca tcaaagccta catgttgatg
 361 actcttcaag gattagaata tttacatcaa cattggatcc tacataggga tctgaaacca
 421 aacaacttgt tgctagatga aaatggagtt ctaaaactgg cagattttgg cctggccaaa
 481 tcttttggga gccccaatag agcttataca catcaggttg taaccaggtg gtatcgggcc
 541 cccgagttac tatttggagc taggatgtat ggtgtaggtg tggacatgtg ggctgttggc
 601 tgtatattag cagagttact tctaagggtt ccttttttgc caggagattc agaccttgat
 661 cagctaacaa gaatatttga aactttgggc acaccaactg aggaacagtg gccggacatg
 721 tgtagtcttc cagattatgt gacatttaag agtttccctg gaataccttt gcatcacatc
 781 ttcagtgcag caggagacga cttactagat ctcatacaag gcttattctt atttaatcca
 841 tgtgctcgaa ttacggccac acaggcactg aaaatgaagt atttcagtaa tcggccaggg
 901 ccaacacctg gatgtcagct gccaagacca aactgtccag tggaaacctt aaaggagcaa
 961 tcaaatccag ctttggcaat aaaaaggaaa agaacagagg ccttagaaca aggaggattg
1021 cccaagaaac taatttttca tcaccatcac catcattaa
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cyclinH Sequence Information

Protein	Human, cyclinH
Accession number	GenBank U11791
Tags	untagged
Native sequence	M1 of native protein is equivalent to M1 of recombinant protein

Recombinant cyclinH amino acid sequence:

```
  1 MYHNSSQKRH WTFSSEEQLA RLRADANRKF RCKAVANGKV LPNDPVFLEP HEEMTLCKYY
 61 EKRLLEFCSV FKPAMPRSVV GTACMYFKRF YLNNSVMEYH PRIIMLTCAF LACKVDEFNV
121 SSPQFVGNLR ESPLGQEKAL EQILEYELLL IQQLNFHLIV HNPYRPFEGF LIDLKTRYPI
181 LENPEILRKT ADDFLNRIAL TDAYLLYTPS QIALTAILSS ASRAGITMES YLSESLMLKE
241 NRTCLSQLLD IMKSMRNLVK KYEPPRSEEV AVLKQKLERC HSAELALNVI TKKRKGYEDD
301 DYVSKKSKHE EEEWTDDDLV ESL
```

Recombinant cyclinH nucleotide sequence:

```
  1 atgtaccaca acagtagtca gaagcggcac tggaccttct ccagcgagga gcagctggca
 61 agactgcggg ctgacgccaa ccgcaaattc agatgcaaag ccgtggccaa cgggaaggtt
121 cttccgaatg atccagtctt tcttgagcct catgaagaaa tgacactctg caaatactat
181 gagaaaaggt tattggaatt ctgttcggtg tttaagccag caatgccaag atctgttgtg
241 ggtacggctt gtatgtattt caaacgtttt tatcttaata actcagtaat ggaatatcac
301 cccaggataa taatgctcac ttgtgcattt ttggcctgca aagtagatga attcaatgta
361 tctagtcctc agtttgttgg aaacctccgg gagagtcctc ttggacagga gaaggcactt
421 gaacagatac tggaatatga actacttctt atacagcaac ttaatttcca ccttattgtc
481 cacaatcctt acagaccatt tgagggcttc ctcatcgact taaagacccg ctatcccata
541 ttggagaatc cagagatttt gaggaaaaca gctgatgact ttcttaatag aattgcattg
601 acggatgctt acctttata cacaccttcc caaattgccc tgactgccat tttatctagt
661 gcctccaggg ctggaattac tatggaaagt tatttatcag agagtctgat gctgaaagag
721 aacagaactt gcctgtcaca gttactagat ataatgaaaa gcatgagaaa cttagtaaag
781 aagtatgaac cacccagatc tgaagaagtt gctgttctga aacagaagtt ggagcgatgt
841 cattctgctg agcttgcact taacgtaatc acgaagaaga ggaaaggcta tgaagatgat
901 gattacgtct caaagaaatc caaacatgag gaggaagaat ggactgatga cgacctggta
961 gaatctctct aa
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MAT1 Sequence Information

Protein Human, MAT1

Accession number GenBank X87843

Tags *N*-terminal GST

Native sequence M1 of native protein is equivalent to M231 of recombinant protein

Recombinant MAT1 amino acid sequence:

```
   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MDDQGCPRCK
 241 TTKYRNPSLK LMVNVCGHTL CESCVDLLFV RGAGNCPECG TPLRKSNFRV QLFEDPTVDK
 301 EVEIRKKVLK IYNKREEDFP SLREYNDFLE EVEEIVFNLT NNVDLDNTKK KMEIYQKENK
 361 DVIQKNKLKL TREQEELEEA LEVERQENEQ RRLFIQKEEQ LQQILKRKNK QAFLDELESS
 421 DLPVALLLAQ HKDRSTQLEM QLEKPKPVKP VTFSTGIKMG QHISLAPIHK LEEALYEYQP
 481 LQIETYGPHV PELEMLGRLG YLNHVRAASP QDLAGGYTSS LACHRALQDA FSGLFWQPS
```

Recombinant MAT1 nucleotide sequence:

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggaagttc tgttccaggg gcccgaattc atggacgatc agggttgccc tcggtgtaag
 721 accaccaaat atcggaaccc ctccttgaag ctgatggtga atgtgtgcgg acacactctc
 781 tgtgaaagtt gtgtagattt actgtttgtg agaggagctg gaaactgccc tgagtgtggt
 841 actccactca gaaagagcaa cttcagggta caactctttg aagatcccac tgttgacaag
 901 gaggttgaga tcaggaaaaa agtgctaaag atatacaata aaagggaaga agattttcct
 961 agtctaagag aatacaatga tttcttggaa gaagtggaag aaattgtttt caacttgacc
1021 aacaatgtgg atttggacaa caccaaaaag aaaatggaga tataccaaaa ggaaaacaaa
1081 gatgttattc agaaaaataa attaaagctg actcgagaac aggaagaact ggaagaagct
1141 ttagaagtgg aacgacagga aaatgaacaa agaagattat ttatacaaaa agaagaacaa
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1321 caacttgaga aacccaaacc tgtaaaacca gtgacgtttt ccacaggcat caaaatgggt
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1441 ctgcagatag agacatatgg accacatgtt cctgagcttg agatgctagg aagacttggg
1501 tatttaaacc atgtcagagc tgcctcacca caggaccttg ctggaggcta tacttcttct
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Reviewed and approved by site quality representative.