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Certificate of Analysis

Cdk3/cyclinE, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-487, 14-487-K, 14-487M
Parent Lot # D8JN017U

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: *C*-terminal 6His-tagged recombinant human full-length cdk3 co-expressed with *N*-terminal GST-tagged recombinant human full-length cyclin E. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 73% by SDS-PAGE and Coomassie blue staining. cdk3 MW = 36kDa, cyclinE MW = 74kDa.

Specific Activity (Parent lot# D8JN017U): 620U/mg, where one unit of cdk3/cyclinE, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml histone H1 per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.016mg/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.

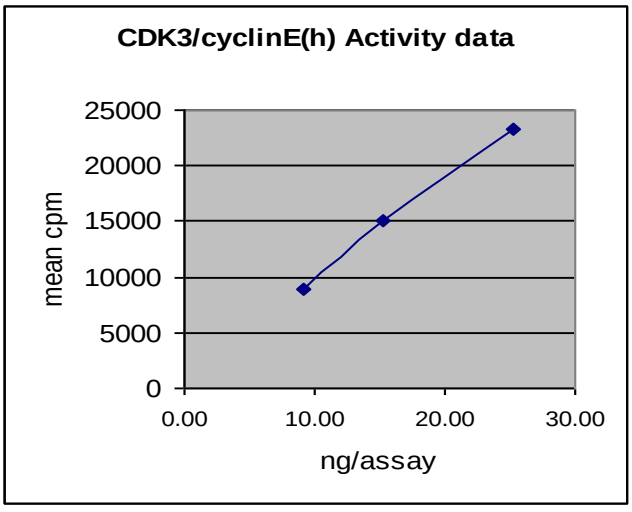
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

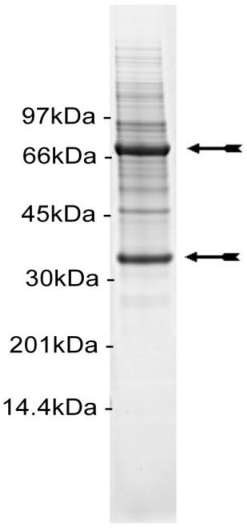
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 9–25ng of this lot of enzyme phosphorylated 0.1mg/ml histone H1 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 cdk3 with the translated sequence listed on page three. Confirmed identity as cyclinE with the translated sequence listed on page four.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of cdk3/cyclinE, active

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Histone H1:** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock in 20mM MOPS/NaOH pH7.0. Add 2.5µl of stock per assay point.
3. **cdk3/cyclinE, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9–25ng 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 **histone H1**.
3. Add **2.5µl (9–25ng) cdk3/cyclinE, 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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cdk3 Sequence Information

Protein human cdk3

Tags *C*-terminal 6His

Native sequence H305 of the recombinant protein is equivalent to H305, the last amino acid, of human cdk3

Accession number GenBank X66357

Recombinant cdk3 amino acid sequence:

```
  1 MDMFQKVEKI GEGTYGVVYK AKNRETGQLV ALKKIRLDLE MEGVPSTAIR EISLLKELKH
 61 PNIVRLLDVV HNERKLYLVF EFLSQDLKKY MDSTPGSELP LHLIKSYLFQ LLQGVSFCHS
121 HRVIHRDLKP QNLLINELGA IKLADFGLAR AFGVPLRTYT HEVVTLWYRA PEILLGSKFY
181 TTAVDIWSIG CIFAEMVTRK ALFPGDSEID QLFRIFRMLG TPSEDTWPGV TQLPDYKGSF
241 PKWTRKGLEE IVPNLEPEGR DLLMQLLQYD PSQRITAKTA LAHPYFSSPE PSPAARQYVL
301 QRFRHHHHHH
```

Recombinant cdk3 nucleotide sequence:

```
  1 atggatatgt tccagaaggt agagaagatc ggagagggca cctatggggt ggtgtacaag
 61 gccaagaaca gggagacagg gcagctggtg gccctgaaga agatcagact ggatttggag
121 atggaggggg tcccaagcac tgccatcagg gagatctcgc tgctcaagga actgaagcac
181 cccaacatcg tccgactgct ggacgtggtg cacaacgaga ggaagctcta tctggtgttt
241 gagttcctca gccaggacct gaagaagtac atggactcca ccccaggctc agagctcccc
301 ctgcacctca tcaagagcta cctcttccag ctgctgcagg gggtgagttt ctgccactca
361 catcgggtca tccaccgaga cctgaagccc cagaacctgc tcatcaatga gttgggtgcc
421 atcaagctgg ctgacttcgg cctggctcgc gccttcgggg tgcccctgcg cacctacacc
481 catgaggtgg tgacactgtg gtatcgcgcc cccgagattc tcttgggcag caagttctat
541 accacagctg tggatatctg gagcattggt tgcatctttg cagagatggt gactcgaaaa
601 gccctgtttc ctggtgactc tgagattgac cagctctttc gtatctttcg tatgctgggg
661 acacccagcg aagacacatg gcccggggtc acccagctgc ctgactataa gggcagcttc
721 cctaagtgga ccaggaaggg actggaagag attgtgccca atctggagcc agagggcagg
781 gacctgctca tgcaactcct gcagtatgac cccagccagc ggatcacagc caagactgcc
841 ctggcccacc cgtacttctc atcccctgag ccctccccag ctgcccgcca gtatgtgctg
901 cagcgattcc gccatcacca tcaccatcat tga
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CyclinE Sequence Information

Protein	human cyclinE
Tags	*N*-terminal GST
Native sequence	M231 of the recombinant protein is equivalent to M1 of human cyclinE
Accession number	GenBank NM_001238

Recombinant cyclinE 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 MPRERRERDA
241 KERDTMKEDG GAEFSARSRK RKANVTVFLQ DPDEEMAKID RTARDQCGSQ PWDNNAVCAD
301 PCSLIPTPDK EDDDRVYPNS TCKPRIIAPS RGSPLPVLSW ANREEVWKIM LNKEKTYLRD
361 QHFLEQHPLL QPKMRAILLD WLMEVCEVYK LHRETFYLAQ DFFDRYMATQ ENVVKTLLQL
421 IGISSLFIAA KLEEIYPPKL HQFAYVTDGA CSGDEILTME LMIMKALKWR LSPLTIVSWL
481 NVYMQVAYLN DLHEVLLPQY PQQIFIQIAE LLDLCVLDVD CLEFPYGILA ASALYHFSSS
541 ELMQKVSGYQ WCDIENCVKW MVPFAMVIRE TGSSKLKHFR GVADEDAHNI QTHRDSLDLL
601 DKARAKKAML SEQNRASPLP SGLLTPPQSG KKQSSGPEMA
```

Recombinant cyclinE 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 atgccgaggg agcgcaggga gcgggatgcg
 721 aaggagcggg acaccatgaa ggaggacggc ggcgcggagt tctcggctcg ctccaggaag
 781 aggaaggcaa acgtgaccgt ttttttgcag gatccagatg aagaaatggc caaaatcgac
 841 aggacggcga gggaccagtg tgggagccag ccttgggaca ataatgcagt ctgtgcagac
 901 ccctgctccc tgatccccac acctgacaaa gaagatgatg accgggttta cccaaactca
 961 acgtgcaagc ctcggattat tgcaccatcc agaggctccc cgctgcctgt actgagctgg
1021 gcaaatagag aggaagtctg gaaaatcatg ttaaacaagg aaaagacata cttaagggat
1081 cagcactttc ttgagcaaca ccctcttctg cagccaaaaa tgcgagcaat tcttctggat
1141 tggttaatgg aggtgtgtga agtctataaa cttcacaggg agacctttta cttggcacaa
1201 gatttctttg accggtatat ggcgacacaa gaaaatgttg taaaaactct tttacagctt
1261 attgggattt catctttatt tattgcagcc aaacttgagg aaatctatcc tccaaagttg
1321 caccagtttg cgtatgtgac agatggagct tgttcaggag atgaaattct caccatggaa
1381 ttaatgatta tgaaggccct taagtggcgt ttaagtcccc tgactattgt gtcctggctg
1441 aatgtataca tgcaggttgc atatctaaat gacttacatg aagtgctact gccgcagtat
1501 ccccagcaaa tctttataca gattgcagag ctgttggatc tctgtgtcct ggatgttgac
1561 tgccttgaat ttccttatgg tatacttgct gcttcggcct tgtatcattt ctcgtcatct
1621 gaattgatgc aaaaggtttc agggtatcag tggtgcgaca tagagaactg tgtcaagtgg
1681 atggttccat ttgccatggt tataagggag acggggagct caaaactgaa gcacttcagg
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1741 ggcgtcgctg atgaagatgc acacaacata cagacccaca gagacagctt ggatttgctg
1801 gacaaagccc gagcaaagaa agccatgttg tctgaacaaa atagggcttc tcctctcccc
1861 agtgggctcc tcaccccgcc acagagcggt aagaagcaga gcagcgggcc ggaaatggcg
1921 tga
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Reviewed and approved by site quality representative.