

Certificate of Analysis

Cdk9/cyclin T1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-685, 14-685-K, 14-685M

Parent Lot # WAB0200

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, full-length recombinant, human cdk9 co-expressed with untagged, full-length human cyclin T1. Both expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 43% by SDS-PAGE and Coomassie blue staining. cdk9 MW = 44kDa and cyclinT1 MW = 80.79kDa.

Specific Activity (Parent lot# WAB0200): 111U/mg, where one unit of cdk9/cyclin T1, active activity is defined as 1nmol phosphate incorporated into 100µM PDKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.69mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

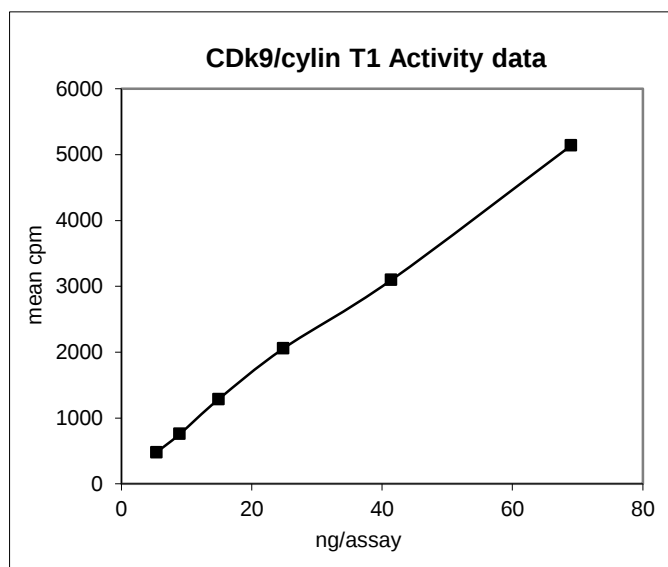
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

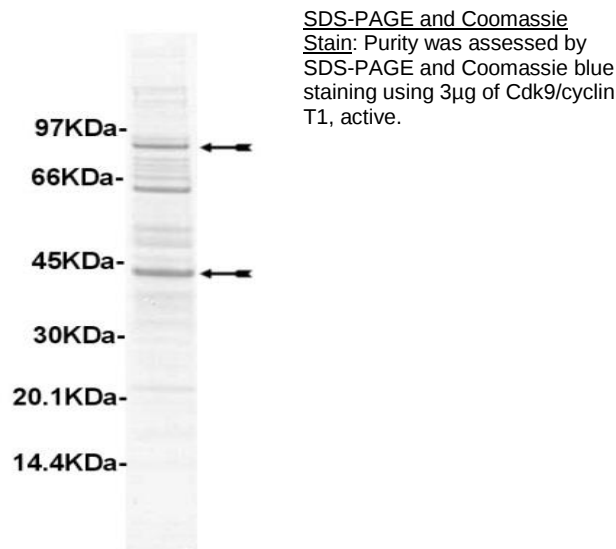
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5–69ng of this lot of enzyme phosphorylated 100µM PDKtide 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 Cdk9 with the translated native sequence listed on page three and cyclin T1 with the translated native sequence listed on page four.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
- 2. PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC):** Use at a final assay concentration of 100µM. Prepare a 1mM stock. Add 2.5µl of stock per assay point.
- 3. Cdk9/cyclin T1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5–69ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]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 **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC)**.
3. Add **2.5µl (5–69ng) cdk9/cyclin T1, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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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Cdk9 Sequence Information

<u>Protein</u>	human cdk9
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human cdk9
<u>Accession number</u>	GenBank AF517840

Recombinant cdk9 amino acid sequence:

```

1 MAKQYDSVEC PFCDEVSKYE KLAIGQGTF GEVFKARHRK TGQKVALKKV LMENEKEGFP
61 ITALREIKIL QLLKHENVVN LIEICRTKAS PYNRCKGSIY LVDFDFCEHDL AGLLSNVLVK
121 FTLSEIKRVM QMLLNGLYYI HRNKILHRDM KAANVLITRD GVLKLADFGL ARAFSLAKNS
181 QPNRYTNRV TLWYRPPPELL LGERDYGPPI DLWGAGCIMA EMWTRSPIMQ GNTEQHQLAL
241 ISQLCGSITP EVWPNVDNYE LYEKLELVKG QKRKVKDRK AYVRDPYALD LIDKLLVLDP
301 AQRIDSDDAL NHDFFWSDPM PSDLKGMLST HLTSMFEYLA PPRRKGQSIT QQSTNQSRNP
361 ATTNQTEFER VFHHHHHH

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Recombinant cdk9 nucleotide sequence:

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1 atggcaaagc agtacgactc ggtggagtg cctttttgtg atgaagt ttc caaatacgag
61 aagctcgcca agatcggcca aggcaccttc ggggagggtg tcaaggccag gcaccgcaag
121 accggccaga aggtggctct gaagaagggt ctgatggaaa acgagaagga ggggttcccc
181 attacagcct tgcgggagat caagatcctt cagcttctaa aacacgagaa tgtggtcaac
241 ttgattgaga tttgtcgaac caaagcttcc ccctataacc gctgcaaggg tagtatatac
301 ctggtgttcg acttctgcga gcatgacctt gctgggctgt tgagcaatgt tttggtcaag
361 ttcacgctgt ctgagatcaa gagggtgatg cagatgctac ttaacggcct ctactacatc
421 cacagaaaca agatcctgca tagggacatg aaggctgcta atgtgcttat cactcgtgat
481 ggggtcctga agctggcaga ctttgggctg gccggggcct tcagcctggc caagaacagc
541 cagcccaacc gctacaccaa ccgtgtgggt acactctggt accggccccc ggagctgttg
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901 gccagcgca tcgacagcga tgacgcctc aaccacgact tcttctggtc cgaccccatg
961 ccctccgacc tcaagggtcat gctctccacc cacctgacgt ccatgttcga gtacttggca
1021 ccaccgcgc ggaaggcgag ccagatcacc cagcagtcca ccaaccagag tcgcaatccc
1081 gccaccacca accagacgga gtttgagcgc gtcttccatc accatcacca tcattga

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cyclin T1 Sequence Information

<u>Protein</u>	human cyclin T1
<u>Tags</u>	untagged
<u>Native sequence</u>	M1 of recombinant protein is equivalent to M1 of human cyclin T1
<u>Accession number</u>	GenBank NM_001240

Recombinant cyclin T1 amino acid sequence:

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1  MEGERKNNNK  RWYFTREQLE  NSPSRRFGVD  PDKELSYRQQ  AANLLQDMGQ  RLNVSQLTIN
61  TAIVYMRHFY  MIQSFTQFPG  NSVAPAALFL  AAKVEEQPKK  LEHVIKVAHT  CLHPQESLPD
121 TRSEAYLQQV  QDLVILESII  LQTLGFELTI  DHPHTHVVKC  TQLVRASKDL  AQTSYFMATN
181 SLHLTTFSLQ  YTPPVVACVC  IHLACKWSNW  EIPVSTDGKH  WWEYVDATVT  LELLDELTHE
241 FLQILEKTPN  RLKRIWNWRA  CEAAKKTKAD  DRGTDEKTSE  QTILNMISQS  SSDTTIAGLM
301 SMSTSTTSAV  PSLPVSEESS  SNLTSVEMLP  GKRWLSSQPS  FKLEPTQGHR  TSENALTGV
361 DHSLPQDGSN  AFISQKQNSK  SVPSAKVSLK  EYRAKHAEEL  AAQKRQLENM  EANVKSQYAY
421 AAQNLLSHHD  SHSSVILKMP  IEGSENERP  FLEKADKTAL  KMRIPVAGGD  KAASSKPEEI
481 KMRIKVHAAA  DKHNSVEDSV  TKSREHKEKH  KTHPSNHHHH  HNHHSHKHSH  SQLPVG TGKN
541 RPDGPKHSSQ  TSNLAHKTY  SSSSFSSSS  TRKRGPSSE  GGAVFDHPAK  IAKSTKSSSL
601 NFSFSLPTM  QMPGHSSDT  SGLSFSQPSC  KTRVPHSKLD  KGPTGANGHN  TTQTIDYQDT
661 VNMLHSLLSA  QGVQPTQPTA  FEFVRPYSDY  LNPRSGGISS  RSGNTDKPRP  PPLPSEPPPP
721 LPPLPK

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Recombinant cyclin T1 nucleotide sequence:

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1  atggaggaggag  agaggaagaa  caacaacaaa  cgggtggtatt  tcactcgaga  acagctggaa
61  aatagcccat  cccgtcgttt  tggcgtggac  ccagataaag  aacttttcta  tcgccagcag
121 gcggccaatc  tgcttcagga  catggggcag  cgtcttaacg  tctcacaatt  gactatcaac
181 actgctatag  tatacatgca  tcgattctac  atgattcagt  ccttcacaca  gttccctgga
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301 ttggaacatg  tcatcaaggt  agcacatact  tgtctccatc  ctcaggaatc  ccttcctgat
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421 ttgcagactt  taggctttga  actaacaatt  gatcaccac  atactcatgt  agtaaagtgc
481 actcaacttg  ttcgagcaag  caaggactta  gcacagactt  cttacttcat  ggcaaccaac
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1501 acaaaagacc  gagagcacia  agaaaagcac  aagactcacc  catctaata  tcatcatcat
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1621 cgtcctgggtg  atccaaaaca  tagtagccag  acaagcaact  tagcacataa  aacctatagc

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1801 aattttctct tcccttcact tcctacaatg ggtcagatgc ctgggcatag ctcagacaca
1861 agtggccttt ccttttcaca gcccagctgt aaaactcgtg tccctcattc gaaactggat
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2041 tttgaatttg ttcgtcctta tagtgactat ctgaatcctc ggtctggtgg aatctcctcg
2101 agatctggca atacagacaa accccggcca ccacctctgc catcagaacc tcctccacca
2161 cttccacccc ttcctaagta a
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