

Certificate of Analysis

CDK6/cyclin D3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-519, 14-519-K, 14-519M

Parent Lot # 2261733

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: N-terminal, 6His-tagged full-length human CDK6 complexed with N-terminal GST-tagged full-length human cyclin D3, expressed in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose, activated with CAK, and repurified on Ni²⁺/NTA-agarose. Purity 88.4%. MW = 38kDa (CDK6) and 59kDa (cyclin D3).

Specific Activity (Parent lot# 2261733): 83U/mg, where one unit of CDK6/cyclinD3, 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: 1.298mg/ml of enzyme in 50mM Tris/HCl pH7.5, 270mM sucrose, 150mM NaCl, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 0.1mM EGTA, 0.03% Brij-35. Frozen solution.

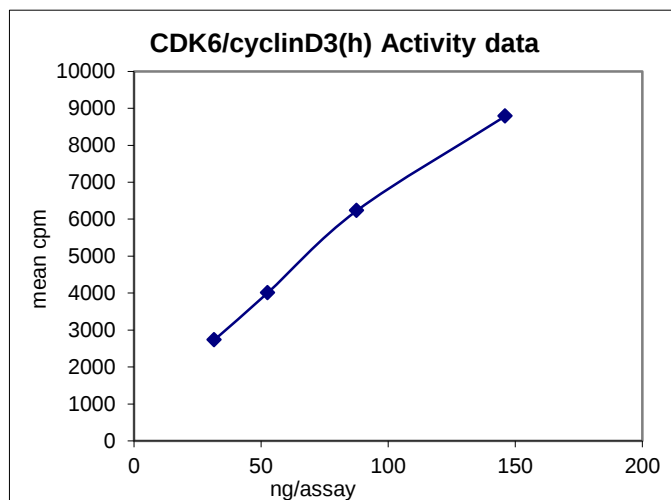
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

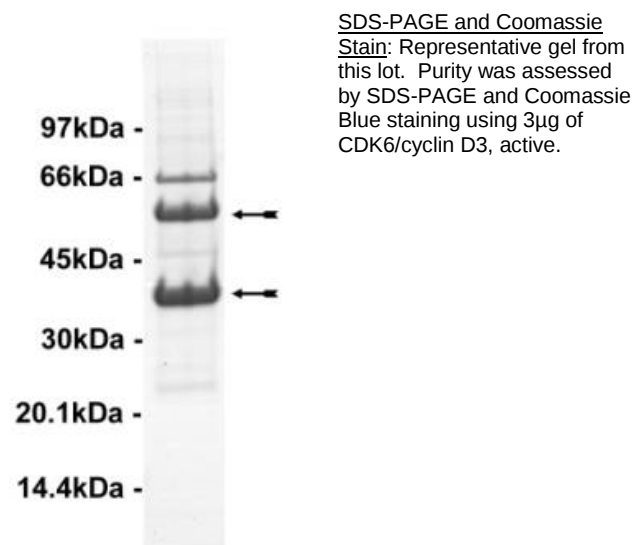
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 32–146ng 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 human CDK6/cyclin D3 with the translated native sequence listed on pages three and four.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA
- 2. Histone H1 substrate:** Use a final assay concentration of 100µg/ml. Make up a 1mg/ml stock. Add 2.5µl of stock per assay point.
- 3. CDK6/cyclin D3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 5% glycerol, 1mM EDTA, 1mg/ml BSA, 0.1% 2-mercaptoethanol, 0.01% Brij-35. Use 32–146ng 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 **histone H1**.
3. Add 5µl dH₂O.
4. Add **2.5µl (32–146ng) CDK6/cyclin D3, active**.
5. Add 10µl 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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CDK6 Sequence Information

<u>Protein</u>	Human CDK6
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the fusion protein is equivalent to M1 of human CDK6
<u>Accession number</u>	GenBank X66365

Recombinant CDK6 amino acid sequence:

```

1  MHHHHHMEK DGLCRADQQY ECVAEIGEGA YGKVFKARDL KNGGRFVALK RVRVQTGEEG
61  MPLSTIREVA VLRHLETFEH PNVVRLFDVC TVSRTDRETK LTLVFEHVDQ DLTTYLDKVP
121 EPGVPTETIK DMMFQLLRGL DFLHSHRVVH RDLKPQNILV TSSGQIKLAD FGLARIYSFQ
181 MALTSVVVTL WYRAPEVLLQ SSYATPVDLW SVGCIFAEMF RRKPLFRGSS DVDQLGKILD
241 VIGLPGEEDW PRDVALPRQA FHKSQAQPIE KFVTDIDELG KDLLKCLTF NPAKRISAYS
301 ALSHPYFQDL ERCKENLDSE LPPSQNTSEL NTA

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Recombinant CDK6 nucleotide sequence:

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1  atgcatcatc accatcacca tatggagaag gacggcctgt gccgcgctga ccagcagtac
61  gaatgcgtgg cggagatcgg ggagggcgcc tatgggaagg tgttcaaggc ccgcgacttg
121 aagaacggag gccgtttcgt ggcgttgaag cgcgtgcggg tgcagaccgg cgaggagggc
181 atgccgtctc ccaccatccg cgaggtggcg gtgctgaggc acctggagac cttcagcac
241 cccaacgtgg tcaggttggt tgatgtgtgc acagtgtcac gaacagacag agaaacaaa
301 ctaactttag tgtttgaaca tgtcgatcaa gacttgacca cttacttgga taaagttcca
361 gagcctggag tgcccactga aaccataaag gatatgatgt ttcagcttct ccgaggtctg
421 gactttcttc attcacaccg agtagtgcat cgcgatctaa aaccacagaa cattctggtg
481 accagcagcg gacaaataaa actcgctgac ttcggccttg ccgcatcta tagtttccag
541 atggctctaa ctcagtggt cgtcacgctg tggtagagag caccgaagt cttgctccag
601 tccagctacg ccaccccggt ggatctctgg agtgttggtg gcatatttgc agaaatgttt
661 cgtagaaagc ctctttttcg tggaaagtca gatgttgatc aactaggaaa aatcttgga
721 gtgattggac tcccaggaga agaagactgg ctagagatg ttgcccttcc caggcaggct
781 tttcattcaa aatctgcccc accaattgag aagtttgtaa cagatatcga tgaactaggc
841 aaagacctac ttctgaagtg tttgacattt aaccagcca aaagaatatc tgcctacagt
901 gccctgtctc acccatactt ccaggacctg gaaaggtgca aagaaaacct ggattccac
961 ctgccgcca gccagaacac ctcggagctg aatacagcct ga

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Cyclin D3 Sequence Information

<u>Protein</u>	Human cyclin D3
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M231 of the fusion protein is equivalent to M1 of human cyclin D3
<u>Accession number</u>	EMBL M90814

Recombinant cyclin D3 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MELLCEGTR
241 HAPRAGPDPR LLGDQRLVLS LLRLEERYVP RASYFQCVQR EIKPHMRKML AYWMLEVCEE
301 QRCEEEVFPL AMNYLDRLYS CVPTRKAQLQ LLGAVCMLLA SKLRETTPLT IEKLCIYTDH
361 AVSPRQLRDW EVLVLGKLLK DLAAVIAHDF LAFILHRLSL PRDRQALVKK HAQTFLLALCA
421 TDYTFAMYPPI SMIATGSIGA AVQGLGACSM SGDELTELLA GITGTEVDCL RACQEQIEAA
481 LRESLREAAQ TSSSPAPKAP RGSSSQGPSQ TSTPTDVTAI HL

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Recombinant cyclin D3 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatatagct caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatatata atggtgatca tgaaccat cctgacttca tgttgatatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggcctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggaagtgc tgttcaggga gcccgaaatt atggagctgc tgtgttgcca aggcaccggg
721 cacgcgcccc gggccggggc ggaccgcgg ctgctggggg accagcgtgt cctgcagagc
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841 gagatcaagc cgcacatgcg gaagatgctg gcttactgga tgctggaggt atgtgaggag
901 cagcgtgtg agagggaagt cttccccctg gccatgaact acctggatcg ctacctgtct
961 tgcgtcccca ccgaaaggc gcagttgcag ctctgggtg cggtctgcat gctgtggcc
1021 tccaagctgc gcgagaccac gccctgacc atcgaaaaac tgtgcatcta caccgaccac
1081 gctgtctctc ccgccagtt gcgggactgg gaggtgctgg tctaggga gctcaagtgg
1141 gacctggctg ctgtgattgc acatgatttc ctggccttca ttctgcaccg gctctctctg
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1261 acagattata ctttgccat gtaccgcca tccatgatcg ccacgggcag cattggggct
1321 gcagtgaag gcctgggtgc ctgctccatg tccggggatg agctcacaga gctgctggca
1381 gggatcactg gactgaagt ggactgcctg cgggcctgtc aggagcagat cgaagctgca
1441 ctcagggaga gcctcaggga agccgctcag accagctcca gccagcgcc caaagcccc
1501 cggggctcca gcagccaagg gccagccag accagcactc ctacagatgt cacagccata
1561 cacctgtag

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