

Certificate of Analysis

Cdk2/cyclinE, active

(Recombinant enzyme expressed in Sf21 Insect cells)

Item # 14-475, 14-475-K, 14-475M

Parent Lot # WAA0025

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 full-length CDK2 in complex with N-terminal GST-tagged, recombinant full-length cyclinE1. Both are expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Combined purity 77.3% by SDS-PAGE and Coomassie blue staining. CDK2 MW = 34kDa, cyclinE1 MW = 74kDa.

Specific Activity (Parent lot# WAA0025): 1898U/mg, where one unit of CDK2/cyclinE1 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: 0.914mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

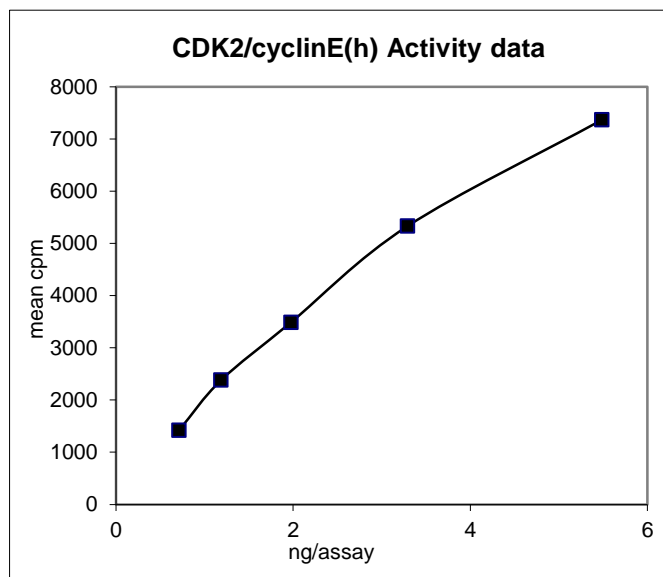
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

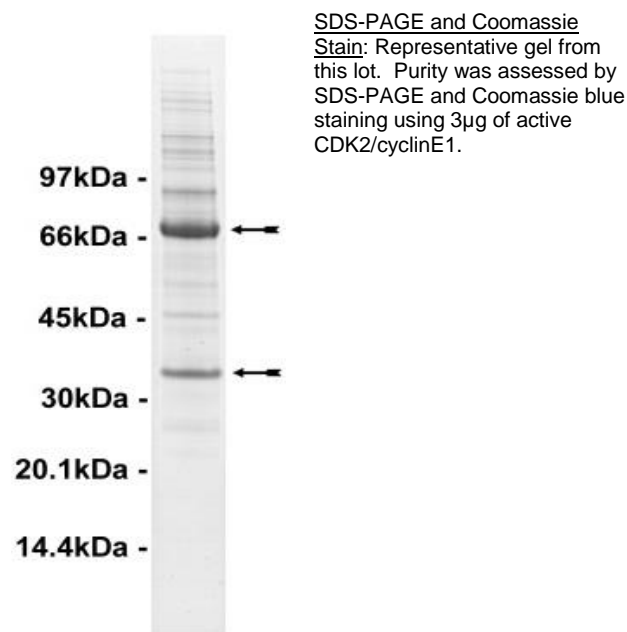
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.7–5.5ng 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 CDK2 with the translated 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:** Use at a final assay concentration of 0.1mg/ml. Make a 1mg/ml stock. Add 2.5µl of stock per assay point.
- 3. CDK2/cyclinE1:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.7–5.5ng 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 **2.5µl (0.7–5.5ng) CDK2/cyclinE1, active**.
4. Make up to 15µl with 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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CDK2/cyclinE1 Sequence Information

<u>Protein</u>	Human CDK2
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human CDK2/cyclinE1
<u>Accession number</u>	EMBL M68520

Recombinant CDK2 amino acid sequence:

```

1  MENFQVEKI  GEGTYGVVYK  ARNKLTGEV  ALKKIRLDTE  TEGVPSTAIR  EISLLKELNH
61  PNIVKLLDVI  HTENKLYLVF  EFLHQDLK  MDASALTGIP  LPLIKSYLFQ  LLQGLAFCHS
121  HRVLHRDLKP  QNLLINTEGA  IKLADFLAR  AFGVPVRTYT  HEVVTWLYRA  PEILLGCKYY
181  STAVDIWSLG  CIFAEMVTRR  ALFPGDSEID  QLFRIFRTL  TPDEVVWPGV  TSMPDYKPSF
241  PKWARQDFSK  VVPLDEDGR  SLLSQMLHYD  PNKRISAKAA  LAHPFFQDVT  KVPVHLRLHH
301  HHHH
  
```

Recombinant CDK2 nucleotide sequence:

```

1  atggagaact  tccaaaaggt  ggaaaagatc  ggagagggca  cgtacggagt  tgtgtacaaa
61  gccagaaaca  agttgacggg  agagtggtg  gcgcttaaga  aaatccgcct  ggacactgag
121  actgagggtg  tgcccagtac  tgccatccga  gagatctctc  tgcttaagga  gcttaacctat
181  cctaattattg  tcaagctgct  ggatgtcatt  cacacagaaa  ataaactcta  cctggttttt
241  gaatttctgc  accaagatct  caagaaattc  atggatgcct  ctgctctcac  tggcattcct
301  cttcccctca  tcaagagcta  tctgttccag  ctgctccagg  gcctagcttt  ctgccattct
361  catcgggtcc  tccaccgaga  ccttaaactc  cagaatctgc  ttattaacac  agagggggcc
421  atcaagctag  cagactttgg  actagccaga  gcttttggag  tccctgttcg  tacttacacc
481  catgagggtg  tgaccctgtg  gtaccgagct  cctgaaatcc  tcctgggctg  caaatattat
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601  gccctattcc  ctggagattc  tgagattgac  cagctcttcc  ggatctttcg  gactctgggg
661  accccagatg  aggtggtgtg  gccaggagtt  acttctatgc  ctgattacaa  gccaaagttt
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781  agcttgttat  cgcaaatgct  gcactacgac  cctaacaagc  ggatttcggc  caaggcagcc
841  ctggctcacc  ctttcttcca  ggatgtgacc  aagccagtac  cccatcttcg  actccatcac
901  catcaccatc  attga
  
```

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CDK2/cyclinE1 Sequence Information

<u>Protein</u>	Human cyclinE1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M231 of recombinant protein is equivalent to M1 of human cyclinE1
<u>Accession number</u>	GenBank NM_001238

Recombinant cyclinE1 amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAI PQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGP EF  MPRRERRERDA
241 KERDTMKEDG  GA EFSARSRK  RKANVTVFLQ  DPDEEMAKID  RTARDQCGSQ  PWDNNAVCAD
301 PCSLIPTPKD  EDDDRVYPNS  TCKPRIAPS  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  TGSSKCLKHFR  GVADEDAHNI  QTHRDSL DLL
601 DKARAKKAML  SEQNRASPLP  SGLLTPPQSG  KKQSSGPEMA
  
```

Recombinant cyclinE1 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaagaa  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  tgaatgctg  aaaatgctcg  aagatcgttt  atgtcataaa
421  acatatataa  atggtgatca  tgtaaccat  cctgacttca  tgttgtatga  cgctcttgat
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601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagtgc  tgttcagggg  gcccaattc  atgccgaggg  agcgcagggg  gcgggtagcg
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1321 caccagtttg  cgtatgtgac  agatggagct  tgttcaggag  atgaaattct  caccatggaa
1381 ttaatgatta  tgaaggccct  taagtggcgt  ttaagtcccc  tgactattgt  gtcttgctg
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1681 atggttccat  ttgccatgg  tataaggag  acggggagct  caaaactgaa  gcacttcagg
1741 ggcgtcgctg  atgaagatgc  acacaacata  cagaccaca  gagacagctt  ggatttgcctg
  
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```
1801 gacaaagccc gagcaaagaa agccatgttg tctgaacaaa atagggttc tcctctcccc  
1861 agtgggctcc tcaccccgcc acagagcggc aagaagcaga gcagcgggcc ggaaatggcg  
1921 tga
```

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