eurofins | Discovery Services

Certificate of Analysis

cdk1/cyclinB, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-450, 14-450-K, 14-450M
Parent Lot # 25729U

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 human full length cdk1, and *N*-terminal GST-tagged human full length cyclinB. These are expressed individually by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose and GST-agarose, respectively. The cdk1 is then activated using CAK and repurified by Q Sepharose and Ni^{2+}/NTA-agarose. They are then complexed *in vitro*. Combined purity 72% by SDS-PAGE and Coomassie blue staining. cdk1 MW = 35kDa, cyclinB MW = 75kDa.

Specific Activity (Parent lot# 25729U): 942U/mg, where one unit of cdk1/cyclinB 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: 2.332mg/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.

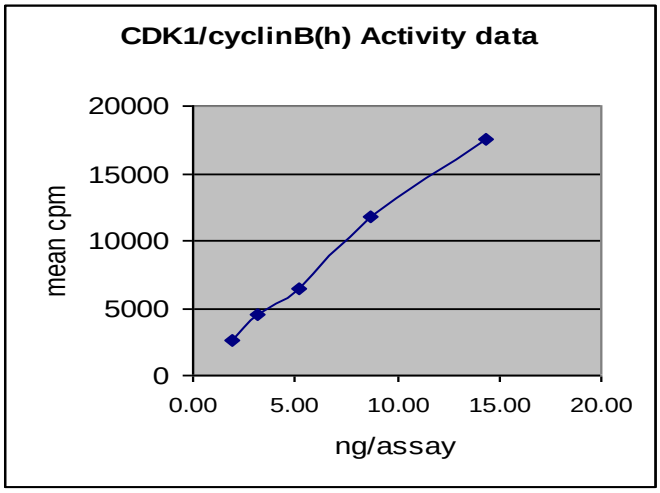
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

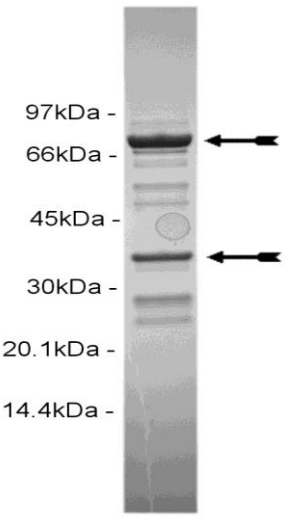
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.89–14.38ng 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 product identity as cdk1 with the translated sequence listed on page three. Confirmed product identity as cyclinB with the translated sequence listed on pages four and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of cdk1/cyclinB.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

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 and add 2.5µl of stock per assay point.
3. **cdk1/cyclinB, active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml Use 1.89–14.38ng 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 (1.89–14.38ng) cdk1/cyclin B, 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.

eurofins | Discovery Services

Certificate of Analysis

cdk1 Sequence Information

Protein human cdk1

Tags *C*-terminal 6His

Native sequence M1 of the recombinant protein is equivalent to M1 of human cdk1

Accession number GenBank NM_001786

Recombinant cdk1 amino acid sequence:

```
  1 MEDYTKIEKI GEGTYGVVYK GRHKTTGQVV AMKKIRLESE EEGVPSTAIR EISLLKELRH
 61 PNIVSLQDVL MQDSRLYLIF EFLSMDLKKY LDSIPPGQYM DSSLVKSYLY QILQGIVFCH
121 SRRVLHRDLK PQNLLIDDKG TIKLADFGLA RAFGIPIRVY THEVVTLWYR SPEVLLGSAR
181 YSTPVDIWSI GTIFAELATK KPLFHGDSEI DQLFRIFRAL GTPNNEVWPE VESLQDYKNT
241 FPKWKPGSLA SHVKNLDENG LDLLSKMLIY DPAKRISGKM ALNHPYFNDL DNQIKKMHHH
301 HHH
```

Recombinant cdk1 nucleotide sequence:

```
  1 atggaagatt ataccaaaat agagaaaatt ggagaaggta cctatggagt tgtgtataag
 61 ggtagacaca aaactacagg tcaagtggta gccatgaaaa aaatcagact agaaagtgaa
121 gaggaagggg ttcctagtac tgcaattcgg gaaatttctc tattaaagga acttcgtcat
181 ccaaatatag tcagtcttca ggatgtgctt atgcaggatt ccaggttata tctcatcttt
241 gagtttcttt ccatggatct gaagaaatac ttggattcta tccctcctgg tcagtacatg
301 gattcttcac ttgttaagag ttatttatac caaatcctac aggggattgt gttttgtcac
361 tctagaagag ttcttcacag agacttaaaa cctcaaaatc tcttgattga tgacaaagga
421 acaattaaac tggctgattt tggccttgcc agagcttttg gaatacctat cagagtatat
481 acacatgagg tagtaacact ctggtacaga tctccagaag tattgctggg gtcagctcgt
541 tactcaactc cagttgacat ttggagtata ggcaccatat ttgctgaact agcaactaag
601 aaaccacttt tccatgggga ttcagaaatt gatcaactct tcaggatttt cagagctttg
661 ggcactccca ataatgaagt gtggccagaa gtggaatctt tacaggacta taagaataca
721 tttcccaaat ggaaaccagg aagcctagca tcccatgtca aaaacttgga tgaaaatggc
781 ttggatttgc tctcgaaaat gttaatctat gatccagcca aacgaatttc tggcaaaatg
841 gcactgaatc atccatattt taatgatttg gacaatcaga ttaagaagat gcatcaccat
901 caccatcatt ag
```

eurofins | Discovery Services

Certificate of Analysis

cyclinB Sequence Information

Protein human cyclinB

Tags *N*-terminal GST

Native sequence M231 of the recombinant protein is equivalent to M1 of human cyclinB

Accession number EMBL M25753.

Recombinant cyclinB 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 MALRVTRNSK
241 INAENKAKIN MAGAKRVPTA PAATSKPGLR PRTALGDIGN KVSEQLQAKM PMKKEAKPSA
301 TGKVIDKKLP KPLEKVPMLV PVPVSEPVPE PEPEPEPEPV KEEKLSPEPI LVDTASPSPM
361 ETSGCAPAEE DLCQAFSDVI LAVNDVDAED GADPNLCSEY VKDIYAYLRQ LEEEQAVRPK
421 YLLGREVTGN MRAILIDWLV QVQMKFRLLQ ETMYMTVSII DRFMQNNCVP KKMLQLVGVT
481 AMFIASKYEE MYPPEIGDFA FVTDNTYTKH QIRQMEMKIL RALNFGLGRP LPLHFLRRAS
541 KIGEVDVEQH TLAKYLMELT MLDYDMVHFP PSQIAAGAFC LALKILDNGE WTPTLQHYLS
601 YTEESLLPVM QHLAKNVVMV NQGLTKHMTV KNKYATSKHA KISTLPQLNS ALVQDLAKAV
661 AKV
```

Recombinant cyclinB 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 atggcgctcc gagtcaccag gaactcgaaa
 721 attaatgctg aaaataaggc gaagatcaac atggcaggcg caaagcgcgt tcctacggcc
 781 cctgctgcaa cctccaagcc cggactgagg ccaagaacag ctcttgggga cattggtaac
 841 aaagtcagtg aacaactgca ggccaaaatg cctatgaaga aggaagcaaa accttcagct
 901 actggaaaag tcattgataa aaaactacca aaacctcttg aaaaggtacc tatgctggtg
 961 ccagtgccag tgtctgagcc agtgccagag ccagaacctg agccagaacc tgagcctgtt
1021 aaagaagaaa aactttcgcc tgagcctatt ttggttgata ctgcctctcc aagcccaatg
1081 gaaacatctg gatgtgcccc tgcagaagaa gacctgtgtc aggctttctc tgatgtaatt
1141 cttgcagtaa atgatgtgga tgcagaagat ggagctgatc caaacctttg tagtgaatat
1201 gtgaaagata tttatgctta tctgagacaa cttgaggaag agcaagcagt cagaccaaaa
1261 tacctactgg gtcgggaagt cactggaaac atgagagcca tcctaattga ctggctagta
1321 caggttcaaa tgaaattcag gttgttgcag gagaccatgt acatgactgt ctccattatt
1381 gatcggttca tgcagaataa ttgtgtgccc aagaagatgc tgcagctggt tggtgtcact
1441 gccatgttta ttgcaagcaa atatgaagaa atgtaccctc cagaaattgg tgactttgct
1501 tttgtgactg acaacactta tactaagcac caaatcagac agatggaaat gaagattcta
1561 agagctttaa actttggtct gggtcggcct ctacctttgc acttccttcg gagagcatct
1621 aagattggag aggttgatgt cgagcaacat actttggcca aatacctgat ggaactaact
1681 atgttggact atgacatggt gcactttcct ccttctcaaa ttgcagcagg agctttttgc
```

Certificate of Analysis

```
1741 ttagcactga aaattctgga taatggtgaa tggacaccaa ctctacaaca ttacctgtca
1801 tatactgaag aatctcttct tccagttatg cagcacctgg ctaagaatgt agtcatggta
1861 aatcaaggac ttacaaagca catgactgtc aagaacaagt atgccacatc gaagcatgct
1921 aagatcagca ctctaccaca gctgaattct gcactagttc aagatttagc caaggctgtg
1981 gcaaaggtgt aa
```

Reviewed and approved by site quality representative.