

## Certificate of Analysis

### Cdc7/cyclin B1, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-025, 16-025-K, 16-025M

Parent Lot # D18CP007N

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 GST-tagged, recombinant, human Cdc7, full length and N-terminal GST-tagged cyclin B1, full length co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Purity 65% (combined) by SDS-PAGE and Coomassie blue staining. MW = 92 kDa (Cdc7) and 75 kDa (cyclin B1)

**Specific Activity (Parent lot# D18CP007N):** 4U/mg, where one unit of Cdc7/cyclin B1 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:** 2.12mg/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.

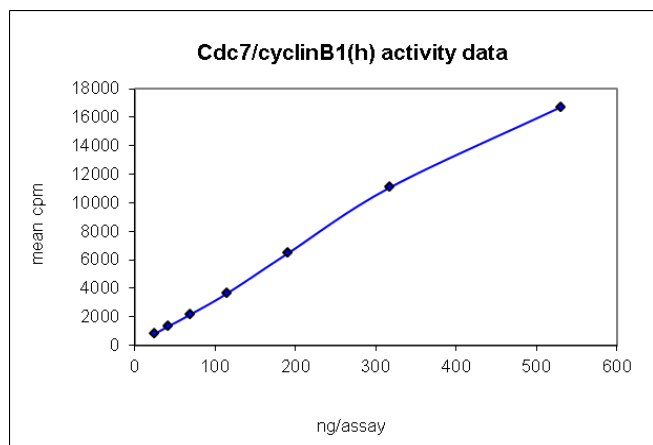
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

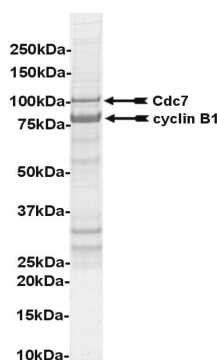
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

#### Quality Control Testing

**Kinase Assay:** 24.73–530.00ng 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 Cdc7/cyclin B1 with the translated sequence listed on pages three to six.



## 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 and add 2.5µl of stock per assay point.
- 3. Cdc7/Cyclin B1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 24.73–530.00ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>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 (24.73–530.00ng) Cdc7/cyclin B1, active.**
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 30 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.

# Certificate of Analysis

## Cdc7, active Sequence Information

<b><u>Protein</u></b>	Human Cdc7
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	M238 of the recombinant protein is equivalent to M1 of human Cdc7
<b><u>Accession number</u></b>	GenBank NM_003503.2

### Recombinant Cdc7 amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKH  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP  EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGP  EFK  KGLRRQVMEA
241  SLGIQMDEPM  AFSPQRDRFQ  AEGSLKNEQ  NFKLAGVKKD  IEKLYEAVPQ  LSNVFKIEDK
301  IGEETFSSVY  LATAQLQVGP  EEKIALKHLI  PTSHPIRIAA  ELQCLTVAGG  QDNVMGVKYC
361  FRKNDHV  VIA  MPYLEHESFL  DILNSLSFQE  VREYMLNLFK  ALKRIHQFGI  VHRDVKPSNF
421  LYNRRLK  KYA  LVDFGLAQGT  HDTKIELLKF  VQSEAQQERC  SQNKSHIITG  NKIPLSGPVP
481  KELDQQST  TK  ASVKRPYTNA  QIQIKQGKDG  KEGSVGLSVQ  RSVFGERNFN  IHSSISHESP
541  AVKLMKQ  SKT  VDVLSRKLAT  KKAISTKVM  NSAVMRKTAS  SCPASLTCDC  YATDKVCSIC
601  LSRRQQV  APR  AGTPGFRAPE  VLTKCPNQTT  AIDMWSAGVI  FLSLLSGRYP  FYKASDDLTA
661  LAQIMTIR  GS  RETIQAAKTF  GKSILCSKEV  PAQDLRKLCE  RLRGMSDSTP  KLTSDIQGHA
721  SHQPAISE  KT  DHKASCLVQT  PPGQYSGNSF  KKGDSNSCEH  CFDEYNTNLE  GWNEVPDEAY
781  DLLDKLL  DLN  PASRITAEAA  LLHPFFKDMS  L

```

### Recombinant Cdc7 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttgaaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttctcta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  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  tgtaaccat  cctgacttca  tgttgtatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcttcc  caaaattagt  ttgttttaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagtgc  tgttccaggg  gcccgaaatt  aaaggcctac  gtcgacaagt  gatggaggcg
721  tctttgggga  ttcagatgga  tgagccaatg  gctttttctc  cccagcgtga  cgggtttcag
781  gctgaaggct  cttaaaaaaa  aaacgagcag  aattttaaac  ttgcagggtg  taaaaaagat
841  attgagaagc  tttatgaagc  tgtaccacag  cttagtaatg  tgtttaagat  tgaggacaaa
901  attggagaag  gcactttcag  ctctgtttat  ttggccacag  cacagttaca  agtaggacct
961  gaagagaaaa  ttgctctaaa  acacttgatt  ccaacaagtc  atcctataag  aatgtagcct
1021  gaacttcagt  gcctaacagt  ggctgggggg  caagataatg  tcatgggagt  taaatactgc
1081  tttaggaaga  atgatcatgt  agttattgct  atgccatata  tggagcatga  gtcgtttttg
1141  gacattctga  attctctttc  ctttcaagaa  gtacgggaat  atatgcttaa  tctgttcaaa
1201  gctttgaaac  gcattcatca  gtttggtatt  gttcaccgtg  atgttaagcc  cagcaatttt
1261  ttatataata  ggcgtctgaa  aaagtatgcc  ttggttagact  ttggtttggc  ccaaggaacc
1321  catgatacga  aatagagct  tcttaaat  gtccagtctg  aagctcagca  ggaaaggtgt

```

## Certificate of Analysis

```
1381 tcacaaaaca aatcccacat aatcacagga aacaagattc cactgagtgg cccagtacct
1441 aaggagctgg atcagcagtc caccacaaaa gcttctgtta aaagacccta cacaaatgca
1501 caaattcaga ttaaacaagg aaaagacgga aaggagggat ctgtaggcct ttctgtccag
1561 cgctctgttt ttggagaaag aaatttcaat atacacagct ccatttcaca tgagagccct
1621 gcagtgaaac tcatgaagca gtcaaagact gtggatgtac tgtctagaaa gttagcaaca
1681 aaaaagaagg ctatttctac aaaagttatg aatagtgctg tgatgaggaa aactgccagt
1741 tcttgcccag ctagcctgac ctgtgactgc tatgcaacag ataaagtttg tagtatttgc
1801 ctttcaaggc gtcagcaggt tgcccctagg gcaggtacac caggattcag agcaccagag
1861 gtcttgacaa agtgcccaa tcaactaca gcaattgaca tgtggtctgc aggtgtcata
1921 tttctttctt tgcttagtgg acgatatcca ttttataaag caagtgatga ttttaactgct
1981 ttggcccaaa ttatgacaat taggggatcc agagaaacta tccaagctgc taaaactttt
2041 gggaaatcaa tattatgtag caaagaagtt ccagcacaag acttgagaaa actctgtgag
2101 agactcaggg gtatggattc tagcactccc aagttaacaa gtgatataca agggcatgct
2161 tctcatcaac cagctatttc agagaagact gaccataaag cttcttgccct cgttcaaca
2221 cctccaggac aatactcagg gaattcattt aaaaaggggg atagtaatag ctgtgagcat
2281 tgttttgatg agtataatac caatttagaa ggctggaatg aggtacctga tgaagcttat
2341 gacctgcttg ataaacttct agatctaaat ccagcttcaa gaataacagc agaagaagct
2401 ttgttgcac cttttttta agatatgagc ttgtga
```

# Certificate of Analysis

## Cyclin B1, active Sequence Information

<b><u>Protein</u></b>	Human cyclin B1
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	M231 of the recombinant protein is equivalent to M1 of human cyclin B1
<b><u>Accession number</u></b>	GenBank NM_031966.3

### Recombinant cyclin B1 amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGP EF  MALRVTRNSK
241  INAENKAKIN  MAGAKRVPTA  PAATSKPGLR  PRTALGDIGN  KVSEQLQAKM  PMKKEAKPSA
301  TGKVIDKKLP  KPLEKVPMLV  PVPVSEPVEPE  PEPEPEPEPV  KEEKLSPEPI  LVDTASPSPM
361  ETSGCAPAEE  DLCQAFSDVI  LAVNDVDAED  GADPNLCSEY  VKDIYAYLRQ  LEEEEQAVRPK
421  YLLGREVTGN  MRAILIDWL V  QVQMKFRLLQ  ETMYMTVSII  DRFMQNNCVP  KKMLQLVGVT
481  AMFIASKYEE  MYPPEIGDFA  FVTDNTYTKH  QIRQMEMKIL  RALNFGLGRP  LPLHFLRRAS
541  KIGEVDVEQH  TLAKYLMELT  MLDYDMVHFP  PSQIAAG AFC  LALKILDNGE  WTPTLQH YLS
601  YTEESLLPVM  QHLAKNVVMV  NQGLTKHMTV  KNKYATSKHA  KISTLPQLNS  ALVQDLAKAV
661  AKV

```

### Recombinant cyclin B1 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttgaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatt  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttcca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  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  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagttc  tgttccaggg  gcccaattc  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  aaaaggatcc  tatgctggtg
961  ccagtgccag  tgtctgagcc  agtgccagag  ccagaacctg  agccagaacc  tgagcctggt
1021  aaagaagaaa  aactttcgcc  tgagcctatt  ttggttgata  ctgcctctcc  aagcccaatg
1081  gaaacatctg  gatgtgcccc  tgcagaagaa  gacctgtgtc  aggccttctc  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  gttgttcgag  gagaccatgt  acatgactgt  ctccattatt
1381  gatcggttca  tgcagaataa  ttgtgtgccc  aagaagatgc  tgcagctggt  tgggtgctact
1441  gccatgttta  ttgcaagcaa  atatgaagaa  atgtaccctc  cagaaattgg  tgactttgct
1501  tttgtgactg  acaacactta  tactaagcac  caaatcagac  agatggaaat  gaagattcta

```

## Certificate of Analysis

```
1561 agagctttaa acttttgtct gggtcggcct ctacctttgc acttccttcg gagagcatct
1621 aagattggag aggttgatgt cgagcaacat actttggcca aataacctgat ggaactaact
1681 atgttgact atgacatggt gcactttcct ctttctcaa ttgcagcagg agctttttgc
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 gcaaagggtg aa
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.