

## Certificate of Analysis

### CDK18/cyclin Y, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-031, 15-031-K, 15-031M

Parent Lot # 220260

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialing runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialing run.

**Product Description:** N-terminal GST-tagged, recombinant, human CDK18 full length and N-terminal GST-tagged, recombinant human cyclin Y full length expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Purity (CDK18 and cyclin Y combined) 72% by SDS-PAGE and Coomassie blue staining. MW = 81 kDa (CDK18) and MW = 67 kDa (cyclin Y)

**Specific Activity (Parent lot# 220260):** 144 U/mg, where one unit of CDK18/cyclin Y activity is defined as 1 nmol phosphate incorporated into 100  $\mu$ M (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE) per minute at 30°C with a final ATP concentration of 100  $\mu$ M.

**Formulation:** 3.85 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

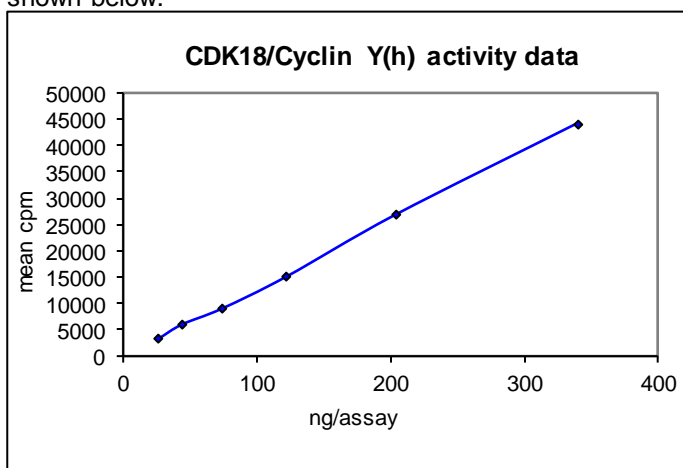
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

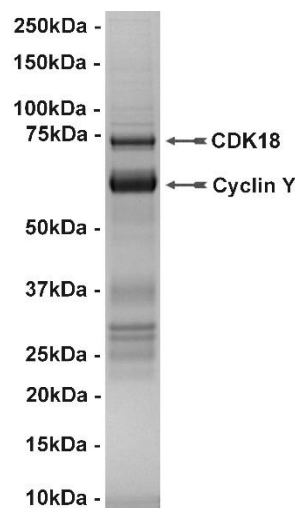
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

#### Quality Control Testing

**Kinase Assay:** 26.4–340 ng of this lot of enzyme phosphorylated 100  $\mu$ M (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE) 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 CDK18/Cyclin Y with the translated sequence listed on page three.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3  $\mu$ g of CDK18/cyclin Y, active.

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### Kinase Assay Protocol

#### Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
- 2. (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE):**  
Use at a final assay concentration of 100  $\mu$ M.  
Prepare a 2 mM stock and add 1.25  $\mu$ l of stock per assay point.
- 3. CDK18/cyclin Y, active:** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 26.4–340 ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

#### Assay Procedure (96 well plate format):

1. Add 5  $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 1.25  $\mu$ l of (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE).
3. Add **2.5  $\mu$ l (26.4–340 ng) CDK18/cyclin Y, active.**
4. Add 6.25  $\mu$ l of dH<sub>2</sub>O.
5. Add 10  $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5  $\mu$ l of 3% phosphoric acid.
8. Transfer a 10  $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4 ml 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 1  $\mu$ l of 30% phosphoric acid.

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### CDK18, active Sequence Information

<b>Protein</b>	Human CDK18
<b>Tags</b>	N-terminal GST
<b>Native sequence</b>	M231 of the recombinant protein is equivalent to M1 of native human CDK18
<b>Accession number</b>	GenBank NM_212502.1. The recombinant protein contains one residue which is in conflict with NM_212502.1. This is T166M and is reported in GenBank BC011526.1.

### Recombinant CDK18 amino acid sequence:

```

1 MSPILGYWKI KGLVQPTLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSR IA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPKSD LVPRGSKELT MIMNMKNFK
241 RRFSLVPR T ETIEESLAEF TEQFNQLHNR RNENLQLGPL GRDPPQECST FSPTDSGEEP
301 GQLSPGVQ FQ RRQNQRFSM EDVSKRLSLP MDIRLPQEF L QKLQMESPD L PKPLSRMSRR
361 ASLSDIGF GK LETYVKLDKL GEGTYATVFK GRSKLMENLV ALKEIRLEHE EGAPCTAIRE
421 VSL LKNL KHA NIVTLHDLIH TDRSLTLVFE YLSDLKQYL DHCNLMMSH NVKIFMFQLL
481 RGLAYCHHR K I LHRDLKPQN LLINER GELK LADFGLARAK SVPTKTYSNE VVTLWYRPPD
541 VLLGST EYST PIDMWGVGCI HYEMATGRPL FPGSTVKEEL HLIFRLLGTP TEETWPGVTA
601 FSEFR TYSFP CYLPQPLINH APRLDTDGIH LLSLLLYES KSRMSAEAL SHSYFRSLGE
661 RVHQLEDTAS IFSLKEIQLQ KDPGYRGLAF QQPGRGKNRR QSIF

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### Recombinant CDK18 nucleotide sequence:

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1 atgtccccta tactaggtta ttgaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcga aaca aaaagtttga attgggttg gagtttcca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttg ggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatat ttaa atggtgatca tgaaccat cctgacttca tgttgatga cgctcttgat
481 gttgtttat acatggacc aatgtgcctg gatgcttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatc caa ggaattgacc atgatcatga acaagatgaa gaactttaag
721 cgccgtttct cctgtcagt gccccgact gagaccattg aagaatcctt ggctgaattc
781 acggagcaat tcaaccagct ccacaaccgg cggaatgaga acttgacgct cggctctctt
841 ggcagagacc ccccagagga gtgcagcacc ttctcccaa cagacagcgg ggaggagccg
901 gggcagctct cccctggcgt gcagttccag cggcggcaga accagcgcgg cttctccatg
961 gaggacgtca gcaagaggct ctctctgccc atggatatcc gcctgccccca ggaattccta
1021 cagaagctac agatggagag cccagacctg cccaagccgc tcagccgcat gtcccgccgg
1081 gcctccctgt cagacattgg ctttgggaaa ctggagacat acgtgaaact ggacaaactg
1141 ggagagggca cctatgcac agtctcaaa gggcgcagca aactgatgga gaaccttggtg
1201 gccctgaaag agatccggct ggagcacgag gagggagcgc cctgcactgc catccgagag
1261 gtgtctctgc tgaagaacct gaagcacgcc aatattgtga ccctgcatga cctcatccac
1321 acagatcggt cctcaccct ggtgtttgag tacctggaca gtgacctgaa gcagtatctg
1381 gaccactgtg ggaacctcat gagcatgcac aacgtcaaga ttttcatggt ccagctgctc

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1441 cggggcctcg cctactgtca ccaccgcaag atcctgcacc gggacctgaa gccccagaac
1501 ctgctcatca acgagagggg ggagctgaag ctggccgact ttggactggc cagggccaag
1561 tcagtgccca caaagactta ctccaatgag gtggtgacc tttgggtacag gcccccgat
1621 gtgctgctgg gatccacaga gtactccacc cccattgata tttggggcgt gggctgcatc
1681 cactacgaga tggccacagg gaggccctc ttcccgggct ccacagtcaa ggaggagctg
1741 cacctcatct ttgcctcct cgggacccc acagaagaga cgtggcccgg cgtgaccgcc
1801 ttctctgagt tccgcaccta cagctccc tgctacctc cgagccgct catcaaccac
1861 gcgcccaggt tggatacggg tggcatccac ctctgagca gcctgctcct gtatgaatcc
1921 aagagtcgca tgtcagcaga ggctgccctg agtcactcct acttccggtc tctgggagag
1981 cgtgtgcacc agcttgaaga cactgcctcc atcttctccc tgaaggagat ccagctccag
2041 aaggacccag gctaccgagg cttggccttc cagcagccag gacgaggaa gaacaggcgg
2101 cagagcatct tctaa

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### cyclin Y, active Sequence Information

**Protein** Human cyclin Y

**Tags** N-terminal GST

**Native sequence** M236 of the recombinant protein is equivalent to M1 of native human cyclin Y

**Accession number** GenBank BC094815.1

### Recombinant cyclin Y amino acid sequence:

```

1 MSPILGYWKI KGLVQPTLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLSSKYIA WPLQGWQATF GGDHPPKSD LVPRGSKEFK GLRRQMGNTT
241 SCCVSSPKL RRNAHSRLES YRPDTLSRE DTGCNLQHIS DRENIDDLNM EFNPSDHPRA
301 STIFLSKSQT DVREKRKSLF INHHPPGQIA RYSSCSTIF LDDSTVSQPN LKYTIKCVL
361 AIYHIKNRD PDGRMLLDIF DENLHPLSKS EVPPDYDKHN PEQKQIYRFV RTLFSAAQLT
421 AECAIVTLVY LERLLTYAEI DICPANWKRI VLGAILLASK VWDDQAVWNV DYCQILKDIT
481 VEDMNERQ FLELLQFNIN VPSSVYAKYY FDLRSLAEAN NLSFPLEPLS RERAHKLEAI
541 SRLCEDKYKD LRRSARKRSA SADNLTLP RW SPAIIS

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### Recombinant cyclin Y nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaagca 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 aaaatgttcg aagatcgttt atgtcataaa
421 acatatataa atggtgatca tgaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc aggcctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat

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```
661 ctggttccgc gtggatcaa ggaattcaaa ggcctacgtc gacaaatggg gaacactacc
721 tcgtgctgcg tgtcgtccag tcccaagctc cggaggaatg cccactccc gctggagtcc
781 taccggccag acacggacct gagccgcgag gacacgggct gcaacctgca gcacatcagc
841 gaccgggaga acatagacga tttgaacatg gaattcaatc cttcagatca tcctcgggcc
901 agcacaatat tcctcagtaa atctcagacg gacgtgagag aaaaacgcaa ggtctcttc
961 attaacatc atcctccagg acaaatagca aggaaataca gttcctgctc caccattttc
1021 ctagatgata gcacagtcag tcaaccaaac ctcaagtata caattaaatg tgtcgtcttt
1081 gcaatatatt atcacatcaa aaacagggac ccagatggaa ggatgctctt agatattttt
1141 gatgaaaatc ttcacctct ttcgaaatcc gaagtgccac cagattatga caaacacaac
1201 ccagagcaga agcagattta ccggttcggt cggacactgt tcagtgtctc tcagctgacg
1261 gctgaaatgt ccatcgtcac cctgggtgtac cttgaaagac ttttaacata cgagagata
1321 gatatctgtc cggccaactg gaagcggatt gttttagggg cgatcctgct ggctccaag
1381 gtgtgggatg accaggctgt atggaatgtg gattactgct agatcctgaa agacatcag
1441 gtggaggaca tgaacgagct agagcgacag tttcttgaat tgctgcagtt caacatcaat
1501 gttccttcca gtgtctatgc caagtattat tttgatctt gttctctggc agaagcgaac
1561 aacctgagct ttcccttgga gccctgagc agggagaggg ctcaacagct tgaggccatc
1621 tctcgcctct gcgaggacaa gtacaaggac ctaagaagat ccgcgaggaa gcgctcagcc
1681 agtgcagaca acctgactct gccccggtgg tccccagcca tcatctctta a
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