

Certificate of Analysis

CDK16/cyclin Y, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-041, 16-041-K, 16-041M

Parent Lot # D17HP013N

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 CDK16 full length and *N*-terminal GST-tagged, recombinant, human cyclin Y full length co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 83kDa (CDK16) and 67kDa (cyclin Y).

Specific Activity (Parent lot# D17HP013N): 330U/mg, where one unit of CDK16/cyclin Y activity is defined as 1nmol phosphate incorporated into 125 μ M PKSPKARKKL per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 2.72mg/ml of enzyme in 50mM Tris/HCl pH8.0, 4mM glutathione, 266mM NaCl, 0.09mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.9mM benzamidine, 0.2mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

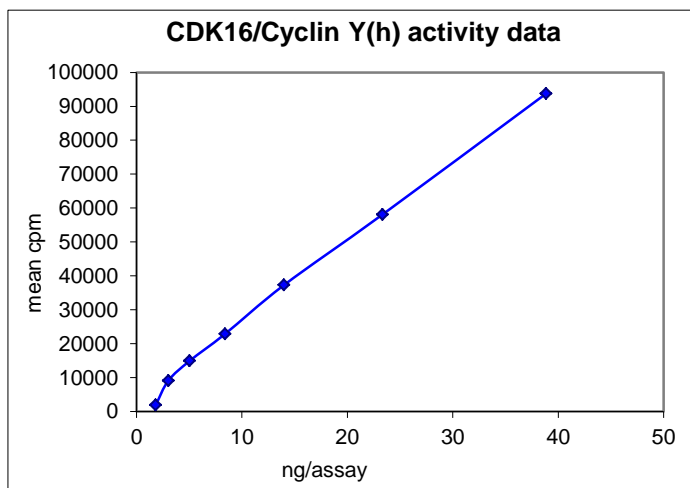
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

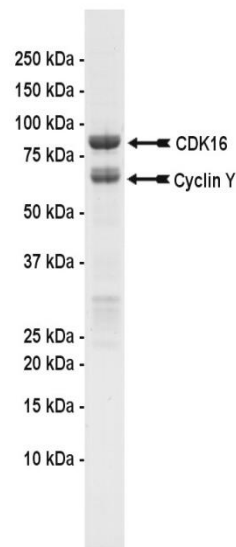
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3–39ng of this lot of enzyme phosphorylated 125 μ M PKSPKARKKL 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 CDK16/cyclin Y with the translated sequences listed on pages three to six.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of CDK16/cyclin Y, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. PKSPKARKKL:** Use at a final assay concentration of 125 μ M. Prepare a 2.5mM stock and add 1.25 μ l of stock per assay point.
- 3. CDK16/cyclin Y, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3–39ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 1500 - 2400cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 1.25 μ l of PKSPKARKKL.
3. Add **2.5 μ l (3–39ng) CDK16/cyclin Y, active.**
4. Add 6.25 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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CDK16 Sequence Information

<u>Protein</u>	Human CDK16
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human CDK16
<u>Accession number</u>	GenBank NM_033018.1

Recombinant CDK16 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFM DRMKKIKRQL
241 SMTLRGGRGI DKTNGAPEQI GLDESGGGGG SDPGEAPTRA APGELRSARG PLSSAPEIVH
301 EDLKMGS DGE SDQASATSSD EVQSPVRVRM RNHPPRKIST EDINKRLSLP ADIRLPEGYL
361 EKLT LNSPI F DKPLSRRLRR VSLSEIGFGK LETYIKLDKL GEGTYATVYK GSKSLTDNLV
421 ALKEIRLEHE EGAPCTAIRE VSLLKDLKHA NIVTLHDI IH TEKSLTLVFE YLDKDLKQYL
481 DDCGNI INMH NVKLF LFQLL RGLAYCHRQK VLHRDLKPQN LLINERGELK LADFLARAK
541 SIPTKYSNE VVTLWYRPPD ILLGSTDYST QIDMWGVGCI FYEMATGRPL FPGSTVEEQL
601 HFIFRILGTP TEETWPGILS NEEFKTYNYP KYRAEALLSH APRLDSGDGAD LLTKLLQFEG
661 RNRISAEDAM KHPFFLSLGE RIHKLPD TTS IFALKEIQ LQ KEASLRSSSM PDSGRPAFRV
721 VDTEF

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Recombinant CDK16 nucleotide sequence:

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1 atgtccccta tactaggtta ttgaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaca aaaagtttga attgggttg gagtttcca atcttcctta ttatattgat
181 ggtgatgta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
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421 acatatttaa atggtgatca tgtaaccat cctgacttca tgttgatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatcaa ggaattcatg gatcggatga agaagatcaa acggcagctg
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1381 acggagaagt ccctcaccct tgtctttgag tacctggaca aggacctgaa gcagtaacctg
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1681 atcctgcttg ggtccacgga ctactccact cagattgaca tgtgggggtg gggctgcatc
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2041 cggatccaca aacttctga cactacttcc atatttgac taaaggagat tcagctacaa
2101 aaggaggcca gccttcggtc ttcgtcgatg cctgactcag gcaggccagc tttccgcgtg
2161 gtggacaccg agttctaa
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Cyclin Y Sequence Information

<u>Protein</u>	Human cyclin Y
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M236 of the recombinant protein is equivalent to M1 of human cyclin Y
<u>Accession number</u>	GenBank BC094815.1

Recombinant cyclin Y 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  LVPRGSKEFK  GLRRQMGNTT
241 SCCVSSSPKL  RRNAHSRLES  YRPDSDLRE  DTGCNLQHIS  DRENIDDLNM  EFNPSDHPRA
301 STIFLSKSQT  DVREKRKSLF  INHHPGQIA  RKYSSCSTIF  LDDSTVSQPN  LKYTIKCVAl
361 AIYYHIKNRD  PDGRMLLDIF  DENLHPLSKS  EVPPDYDKHN  PEQKQIYRFV  RTLFSAAQLT
421 AECAIVTLVY  LERLLTYAEI  DICPANWKRI  VLGAILLASK  VWDDQAVWNV  DYCQILKDIT
481 VEDMNELERQ  FLELLQFNIN  VPSSVYAKYY  FDLRSLAEAN  NLSFPLEPLS  RERAHLEAI
541 SRLCEDKYKD  LRRSARKRSA  SADNLTLPW  SPAIIS

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Recombinant cyclin Y nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttgaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatc  ttgaagaaa  atatgaagag  catttgatg  agcgcgatg  aggtgataaa
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1681 agtgcagaca acctgactct gccccggtgg tccccagcca tcattcttta a

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