

Certificate of Analysis

CDK17/cyclin Y, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-042, 16-042-K, 16-042M

Parent Lot # D17MP006N

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 CDK17 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 80% by SDS-PAGE and Coomassie blue staining. MW = 87kDa (CDK17) and 67kDa (cyclin Y).

Specific Activity (Parent lot# D17MP006N): 489U/mg, where one unit of CDK17/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: 1.11mg/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.

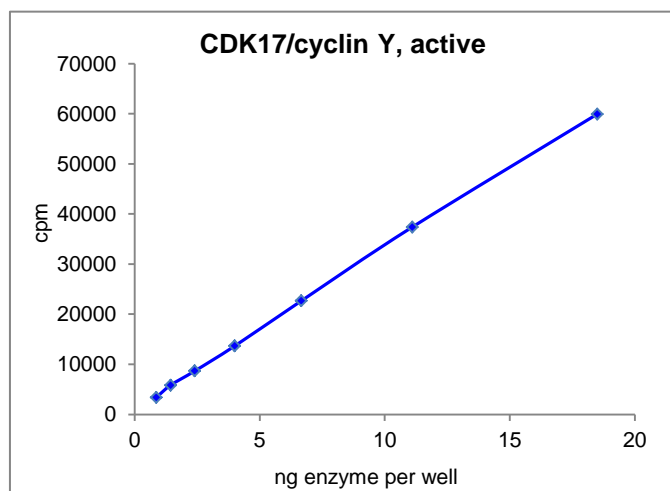
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

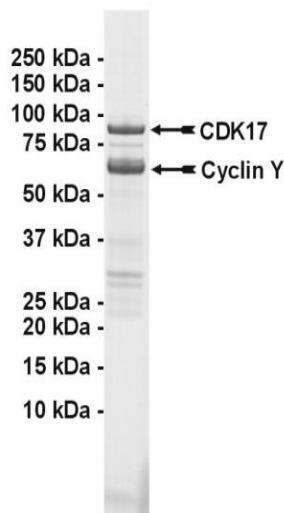
FOR IN VITRO RESEARCH USE ONLY NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.9–18.5ng 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 CDK17/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 CDK17/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. CDK17/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 0.9–18.5ng 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 500 - 800cpm/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 (0.9–18.5ng) CDK17/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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CDK17 Sequence Information

<u>Protein</u>	Human CDK17
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M231 of the recombinant protein is equivalent to M1 of human CDK17
<u>Accession number</u>	GenBank BC033005.1

Recombinant CDK17 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MKKFKRRLSL
241 TLRGSQTIDE SLSELAEQMT IEENSSKDNE PIVKNGRPPT SHSMHSFLHQ YTGSFKKPPL
301 RRPHSVIGGS LGSFMAMPRN GSRLDIVHEN LKMGSDGESD QASGTSSDEV QSPTGVCLRN
361 RIHRRISMED LNKRLSLPAD IRIPDGYLEK LQINSPPFDQ PMSRRSRRAS LSEIGFGKME
421 TYIKLEKLGE GTYATVYKGR SKLTENLVAL KEIRLEHEEG APCTAIREVS LLKDLKHANI
481 VTLHDIVHTD KSLTLVFEYL DKDLKQYMDD CGNIMSMHNV KLFLYQILRG LAYCHRRKVL
541 HRDLKPQNLL INEKGELKLA DFGLARAKSV PTKTYSNEVV TLWYRPPDVL LGSSEYSTQI
601 DMWGVGCIFF EMASGRPLFP GSTVEDELHL IFRLLGTPSQ ETWPGISSNE EFKNYNFPKY
661 KPQPLINHAP RLDSEGIELI TKFLQYESKK RVSAEEAMKH VYFRSLGPRI HALPESVSIF
721 SLKEIQLQKD PGFRNSSYPE TGHGKNRRQS MLF
  
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Recombinant CDK17 nucleotide sequence:

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1 atgtccccta tactaggtta ttgaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatt ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggttg gagtttcca atcttcctta ttatattgat
181 ggtgatgta 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 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 ctggaagttc tgttccaggg gcccaattc atgaaaaaat ttaagagaag gctatccctc
721 aactccgag gaagtcagac tattgatgaa tcattgtctg aattggctga acaaatgact
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841 tctcacagta tgcattcctt cctccaccag tacacaggat ctttcaagaa gccccattg
901 cggagaccac acagtgttat tggagggagc cttggctcct tcatggcaat gccagaaat
961 ggaagcagat tagatattgt tcatgaaaat ctaaaaatgg gatcagatgg tgagagtgac
1021 caagcttctg ggacatcatc tgatgaagtc cagtcaccta caggtgtttg tctcagaat
1081 cgtatacata gacggatctc aatggaggat ttaataaagc ggttatcact gcctgcagac
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1261 acctacatca aattgaaaa gcttggagag ggtacatatg caacagtata taaaggaaga
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1441 gtaaccttac atgacattgt tcacacagat aaatccttga ctttgggtgt tgagtatctg
1501 gataaagacc tgaaacagta catggatgac tgtggaaca tcatgagtat gcacaacgta
  
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1561 aagctgtttc tgtaccaaat tctacgtggt ttggcatatt gccatagaag aaaggtattg
1621 catcgagact tgaaaccaca gaacctcctc attaatgaga aaggagaatt aaagctagca
1681 gattttggac tagcccgagc caagtcagtt cccacaaaga cctactcaa tgaagttgtc
1741 aactatggt accggccacc tgatgtgctt cttggttcct cggagtactc aacacagatt
1801 gacatgtggg gtgttgggtg cattttcttt gaaatggctt ctggaagacc tttattcca
1861 ggatcaaccg tggagatga actgcactta atttccgac tgctaggaac tccatctcag
1921 gaaacttggc caggtatttc ttcaaatgag gagttcaaga actacaactt tccaaaatat
1981 aaaccacagc ctctaattaa ccacgcacc aggttagact ctgaaggaat tgagttgata
2041 acaaaatttc ttcagtatga atctaagaaa agggtttcag ctgaagaggc catgaaacat
2101 gtgtactttc gaagtctggg accaagaata catgctttac cagaaagtgt atcaatattc
2161 agtttgaaag agattcagtt gcaaaaggac cggggttttc gaaattcttc ttatccagag
2221 acaggacatg ggaagaacag aagacagagc atgctctttt aa
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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  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKEFK  GLRRQMGNTT
241  SCCVSSSPKL  RRNAHSRLES  YRPDTLSRE  DTGCNLQHIS  DRENIDDLNM  EFNPSDHPRA
301  STIFLSKSQT  DVREKRKSLF  INHHPPGQIA  RKYSSCSTIF  LDDSTVSQPN  LKYTIKCVAL
361  AIYYHIKNRD  PDGRMLLDIF  DENLHPLSKS  EVPPDYDKHN  PEQKQIYRFV  RTLFSAQQLT
421  AECAIVTLVY  LERLLTYAEI  DICPANWKRI  VLGAILLASK  VWDDQAVWNV  DYCQILKDIT
481  VEDMNELERQ  FLELLQFNIN  VPSSVYAKYY  FDLRSLAEAN  NLSFPLEPLS  RERAHKLEAI
541  SRLCEDKYKD  LRRSARKRSA  SADNLTLPRW  SPAIIS

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Recombinant cyclin Y nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatc  ttgaagaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
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181  ggtgatgta  aattaacaca  gtctatggcc  atcatacggt  atatagctga  caagcacaac
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421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
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1321  gatatctgtc  cggccaactg  gaagcggatt  gttttagggg  cgatcctgct  ggcctccaag
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1441 gtggaggaca tgaacgagct agagcgacag tttcttgaat tgctgcagtt caacatcaat
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1561 aacctgagct ttcccttggg gcccttgagc agggagaggg ctcaacaagct tgaggccatc
1621 tctcgctctt gcgaggaaa gtacaaggac ctaagaagat ccgcgaggaa gcgctcagcc
1681 agtgcagaca acctgactct gcccgggtgg tccccagcca tcatctctta a
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