Eurofins DiscoverX Products France
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Certificate of Analysis

CDK14/cyclin Y, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 15-034, 15-034-K, 15-034M
Parent Lot # 197215

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 6His-tagged, recombinant, human CDK14 full length and *N*-terminal 6His-tagged, recombinant, human cyclin Y full length, co-expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Combined purity 71% by SDS-PAGE and Coomassie blue staining. MW = 54kDa (CDK14) and 44kDa (cyclin Y).

Specific Activity (Parent lot# 197215): 577U/mg, where one unit of CDK14/cyclin Y activity is defined as 1nmol phosphate incorporated into 200µM YRRAAVPPSPSLSRHSSPHQS(p)EDEEE per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.57mg/ml of enzyme in 50mM Tris/HCl pH7.5, 280mM NaCl, 260mM Imidazole, 0.09mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.9mM benzamidine, 0.2mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

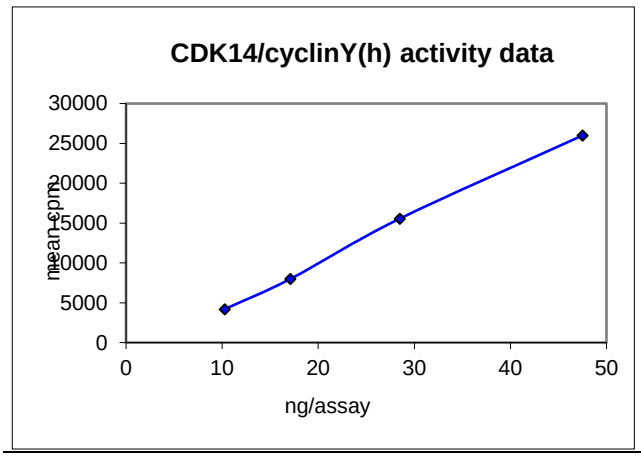
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

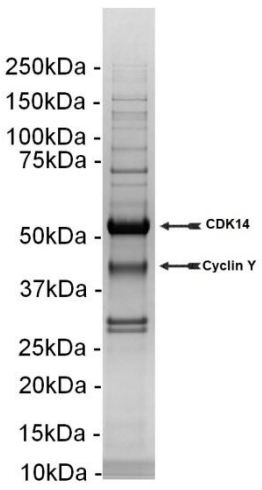
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 10.3–47.5ng of this lot of enzyme phosphorylated 200µM 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 CDK14/cyclin Y with the translated sequence listed on pages three and four.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CDK14/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. **YRRAAVPPSPSLSRHSSPHQS(p)EDEEE**: Use at a final assay concentration of 200µM. Prepare a 2mM stock and add 2.5µl of stock per assay point.
3. **CDK14/cyclin Y, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.04% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 10.3–47.5ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 YRRAAVPPSPSLSRHSSPHQS(p)EDEEE.
3. Add **2.5µl (10.3–47.5ng) CDK14/cyclin Y, 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 dried 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.

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CDK14, active Sequence Information

Protein	Human CDK14
Tags	*N*-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human CDK14
Accession number	GenBank NM_012395.2

Recombinant CDK14 amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MHGYFGCNAA AEPGYSAFVG TPQICVTKMS
 61 TRNCQGMDSV IKPLDTIPED KKVRVQRTQS TFDPFEKPAN QVKRVHSENN ACINFKTSST
121 GKESPKVRRH SSPSSPTSPK FGKADSYEKL EKLGEGSYAT VYKGKSKVNG KLVALKVIRL
181 QEEEGTPFTA IREASLLKGL KHANIVLLHD IIHTKETLTL VFEYVHTDLC QYMDKHPGGL
241 HPDNVKLFLF QLLRGLSYIH QRYILHRDLK PQNLLISDTG ELKLADFGLA RAKSVPSHTY
301 SNEVVTLWYR PPDVLLGSTE YSTCLDMWGV GCIFVEMIQG VAAFPGMKDI QDQLERIFLV
361 LGTPNEDTWP GVHSLPHFKP ERFTLYSSKN LRQAWNKLSY VNHAEDLASK LLQCSPKNRL
421 SAQAALSHEY FSDLPPRLWE LTDMSSIFTV PNVRLQPEAG ESMRAFGKNN SYGKSLSNSK
481 H
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Recombinant CDK14 nucleotide sequence:

```
   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atgcacggtt actttggctg caatgctgct
 121 gcagagcccg gttactctgc cttcgtggga actccacaga tatgtgtcac aaagatgtct
 181 acacggaact gccagggaat ggactcagtg atcaaacccc tggacacaat tcctgaggat
 241 aaaaaagtca gagttcagag gacacagagc acttttgacc catttgagaa accagctaat
 301 caagtaaaga gggtgcattc tgagaacaat gcttgcatta actttaagac ctcctccact
 361 ggcaaagagt cacctaaagt taggcggcac tccagcccca gctcgccaac aagtcccaaa
 421 tttggaaaag ctgactcata tgaaaagctg gaaaaactag gggaaggatc ttatgctaca
 481 gtatacaaag ggaaaagcaa ggtaaatggg aagttggtag ctctgaaggt gatcaggctg
 541 caggaagaag aagggacacc tttcacagct atcagggaag cttctctttt aaaaggacta
 601 aaacatgcta acatagtgct acttcatgac atcatccata ccaaggagac gctgacactt
 661 gtgtttgaat atgtgcacac tgatttatgt cagtacatgg acaagcaccc tgggggggctg
 721 catccagata atgtgaagtt gtttttattt cagttgctgc gaggtctgtc ttacatccac
 781 cagcgttata ttttgcacag agacctgaaa ccacagaacc ttctgatcag tgacacgggg
 841 gagttaaagc tggcagattt cggtcttgca agagcaaaat ccgtccctag ccacacatac
 901 tccaacgaag tggttacctt gtggtacaga cctccagatg tccttctagg ctcaacagaa
 961 tattccacct gccttgacat gtggggagta ggttgcatct ttgttgaaat gatccaagga
1021 gttgctgctt ttccaggaat gaaagacatt caggatcaac ttgaacgaat atttctggtt
1081 cttggaacac caaatgagga cacatggcct ggagttcatt ctttaccaca ttttaagcca
1141 gaacgcttta ccctgtacag ctctaaaaac cttagacaag catggaataa gctcagctat
1201 gtgaaccatg cagaggacct ggcctccaag ctcctacaat gttccccaaa gaacagactg
1261 tcggcacagg ctgccttgag ccacgagtat tttagtgacc tgccgccacg gctatgggaa
1321 ctcaccgaca tgtcttctat ttttactgtc ccaaatgtga gattgcaacc agaagctgga
1381 gaaagcatgc gggcctttgg gaaaaacaat agttatggca aaagtctatc aaacagcaag
1441 cactga
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cyclin Y, active Sequence Information

Protein	Human cyclin Y
Tags	*N*-terminal 6His
Native sequence	M37 of the recombinant protein is equivalent to M1 of human CDK14
Accession number	GenBank BC094815.1

Recombinant cyclin Y amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMGNT TSCCVSSSPK LRRNAHSRLE
 61 SYRPDTDLSR EDTGCNLQHI SDRENIDDLN MEFNPSDHPR ASTIFLSKSQ TDVREKRKSL
121 FINHHPPGQI ARKYSSCSTI FLDDSTVSQP NLKYTIKCVA LAIYYHIKNR DPDGRMLLDI
181 FDENLHPLSK SEVPPDYDKH NPEQKQIYRF VRTLFSAAQL TAECAIVTLV YLERLLTYAE
241 IDICPANWKR IVLGAILLAS KVWDDQAVWN VDYCQILKDI TVEDMNELER QFLELLQFNI
301 NVPSSVYAKY YFDLRSLAEA NNLSFPLEPL SRERAHKLEA ISRLCEDKYK DLRRSARKRS
361 ASADNLTLPR WSPAIIS
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Recombinant cyclin Y nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat ggggaacact
 121 acctcgtgct gcgtgtcgtc cagtcccaag ctccggagga atgcccactc ccggctggag
 181 tcctaccggc cagacacgga cctgagccgc gaggacacgg gctgcaacct gcagcacatc
 241 agcgaccggg agaacataga cgatttgaac atggaattca atccttcaga tcatcctcgg
 301 gccagcacaa tattcctcag taaatctcag acggacgtga gagaaaaacg caagagtctc
 361 ttcattaacc atcatcctcc aggacaaata gcaaggaaat acagttcctg ctccaccatt
 421 ttcctagatg atagcacagt cagtcaacca aacctcaagt atacaattaa atgtgtcgct
 481 cttgcaatat attatcacat caaaaacagg gacccagatg gaaggatgct cttagatatt
 541 tttgatgaaa atcttcaccc tctttcgaaa tccgaagtgc caccagatta tgacaaacac
 601 aacccagagc agaagcagat ttaccggttc gttcggacac tgttcagtgc tgctcagctg
 661 acggctgaat gtgccatcgt caccctggtg taccttgaaa gacttttaac atacgcagag
 721 atagatatct gtccggccaa ctggaagcgg attgttttag gggcgatcct gctggcctcc
 781 aaggtgtggg atgaccaggc tgtatggaat gtggattact gccagatcct gaaagacatc
 841 acggtggagg acatgaacga gctagagcga cagtttcttg aattgctgca gttcaacatc
 901 aatgttcctt ccagtgtcta tgccaagtat tattttgatc ttcgttctct ggcagaagcg
 961 aacaacctga gctttccctt ggagcccctg agcagggaga gggctcacaa gcttgaggcc
1021 atctctcgcc tctgcgagga caagtacaag gacctaagaa gatccgcgag gaagcgctca
1081 gccagtgcag acaacctgac tctgccccgg tggtccccag ccatcatctc ttaa
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Reviewed and approved by site quality representative.