

Certificate of Analysis

CDK4/cyclin D3, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-957, 14-957-K, 14-957M

Parent Lot # D14SP004N

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 CDK4 full length and N-terminal GST-tagged, recombinant, human cyclin D3 full length co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 85% (combined) by SDS-PAGE and Coomassie blue staining. MW = 61kDa (CDK4) and 59kDa (cyclin D3)

Specific Activity (Parent lot# D14SP004N): 129U/mg, where one unit of CDK4/cyclin D3, active activity is defined as 1nmol phosphate incorporated into 0.2mg/ml Rb substrate per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.38mg/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% β-mercaptoethanol. Frozen solution.

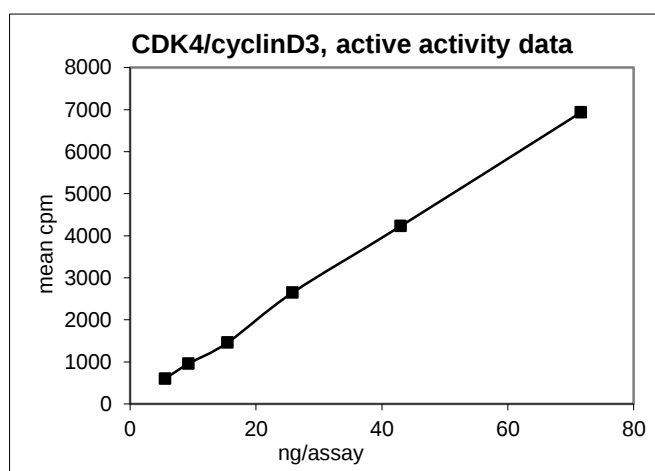
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

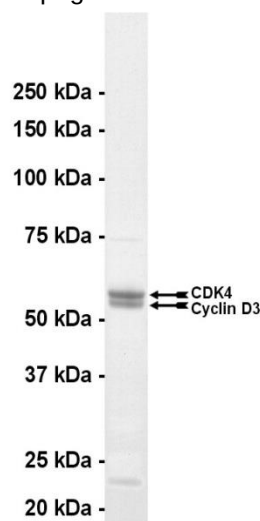
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5–72ng of this lot of enzyme phosphorylated 0.2mg/ml Rb substrate 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 CDK4/cyclin D3 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 1µg of CDK4/cyclin D3, active

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Rb substrate:** Use at a final assay concentration of 0.2mg/ml. Prepare a 2mg/ml stock and add 2.5µl of stock per assay point.
3. **DTT:** Use at a final assay concentration of 20mM. Prepare a 1M stock and add 0.5µl per well.
4. **Tween 20:** Use at a final assay concentration of 0.03%. Prepare a 10% stock and add 0.75µl per well.
5. **BSA:** Use at a final assay concentration of 0.03%. Prepare a 10% stock and add 0.75µl per well.
6. **CDK4/Cyclin D3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% β-mercaptoethanol, 1mg/ml BSA. Use 5–72ng per assay point.
7. **[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 4.35µl of dH₂O.
3. Add 0.5µl of 1M DTT.
4. Add 0.75µl of 10% Tween 20
5. Add 0.75µl of 10% Bovine Serum Albumin (BSA).
6. Add 2.5µl (5–72ng) **CDK4/cyclin D3, active**.
7. Add 2.5µl of Rb substrate.
8. Add 10µl of diluted [γ-³³P]ATP mixture.
9. Incubate for 10 minutes at 30°C.
10. Stop the reaction by adding 5µl of 3% phosphoric acid.
11. Transfer a 10µl aliquot onto the appropriate area of a P30 Filtermat.
12. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
13. Wash the filtermat once for 2 minutes with methanol.
14. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
15. 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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CDK4 Sequence Information

<u>Protein</u>	Human CDK4
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M231 of the recombinant protein is equivalent to M1 of human CDK4.
<u>Accession number</u>	GenBank NM_000075.3

Recombinant CDK4 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKH  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCH  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LEVLFQGPEF  MATSRYEPVA
241  EIGVGAYGT  YKARDPHSGH  FVALKSVRVP  NGGGGGGGLP  ISTVREVAL  RRLEAFEHPN
301  VVRLMDVCA  SRTDREIKVT  LVFEHVDQDL  RTYLDKAPPP  GLPAETIKDL  MRQFLRGLDF
361  LHANCIVHRD  LKPENILVTS  GGTVKLADFG  LARIYSYQMA  LTPVVVTLWY  RAPEVLLQST
421  YATPVDMSV  GCIFAEMFRR  KPLFCGNSEA  DQLGKIFDLI  GLPPEDDWPR  DVSLPRGAFP
481  PRGPRPVQSV  VPMEESGAQ  LLEMLTFNP  HKRISAFRAL  QHSYLHKDEG  NPE

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Recombinant CDK4 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgtat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atatactga  caagcacaac
241  atgttggttg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatTTaa  atggtgatca  tgtaacccat  cctgacttca  tgttgtatga  cgctcttgat
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841  atcagcacag  ttcgtgaggt  ggctttactg  aggcgactgg  aggcctttga  gcatcccaat
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1501  ctgctgctgg  aaatgctgac  ttttaaccca  cacaagcgaa  tctctgcctt  tcgagctctg
1561  cagcactctt  atctacataa  ggatgaagggt  aatccggagt  ga

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Cyclin D3 Sequence Information

Protein	Human cyclin D3
Tags	N-terminal GST
Native sequence	M231 of the recombinant protein is equivalent to M1 of human cyclin D3.
Accession number	GenBank M90814.1

Recombinant cyclin D3 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	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LEVLFQGPEF	MELLCCEGTR
241	HAPRAGPDPR	LLGDQRVLQS	LLRLEERYVP	RASYFQCVR	EIKPHMRKML	AYWMLEVCEE
301	QRCEEEVFPL	AMNYLDRYLS	CVPTRKAQLQ	LLGAVCMLLA	SKLRETTPLT	IEKLCIYTDH
361	AVSPRQLRDW	EVLVLGKLKW	DLAAVIAHDF	LAFILHRLSL	PRDRQALVKK	HAQTFLALCA
421	TDYTFAMYPP	SMIATGSIGA	AVQGLGACSM	SGDELTELLA	GITGTEVDCL	RACQEQIEAA
481	LRESLREAAQ	TSSSPAPKAP	RGSSSQGPSQ	TSTPTDVTAI	HL	

Recombinant cyclin D3 nucleotide sequence:

1	atgtccccta	tactaggtta	ttggaaaatt	aagggccttg	tgcaaccac	tcgacttctt
61	ttggaatata	ttgaagaaaa	atatgaagag	catttgatatg	agcgcgatga	aggtgataaa
121	tggcgaaaca	aaaagtttga	attgggtttg	gagtttccca	atcttcctta	ttatatgtat
181	ggtgatgtta	aattaacaca	gtctatggcc	atcatacgtt	atatactga	caagcacaac
241	atgttggttg	gttgtccaaa	agagcgtgca	gagatttcaa	tgcttgaagg	agcggttttg
301	gatattagat	acggtgtttc	gagaattgca	tatagtaaag	actttgaaac	tctcaaagtt
361	gattttctta	gcaagctacc	tgaaatgctg	aaaatgttcg	aagatcgttt	atgtcataaa
421	acataattta	atggtgatca	tgtaacccat	cctgacttca	tggtgtatga	cgctcttgat
481	gttggtttat	acatggaccc	aatgtgcctg	gatgcgttcc	caaaattagt	ttgttttaaa
541	aaacgtattg	aagctatccc	acaaaattgat	aagtacttga	aatccagcaa	gtatatagca
601	tggcctttgc	agggctggca	agccacgttt	ggtggtggcg	accatcctcc	aaaatcggat
661	ctggaagtgc	tggtccaggg	gcccgaattc	atggagctgc	tgtgttgcca	aggcaccggg
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1561	cacctgtag					

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