

## CDK6/CycD3

cyclin dependent kinase 6

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK6

Synonyms: PLSTIRE

Product No.: 0051-0373-1

Lot: 004

**Description:** Human CDK6, amino acids M<sub>1</sub>-A<sub>326</sub> (as in [NCBI/Protein](#) entry NP\_001250.1), N-terminal GST fusion protein with a Thrombin cleavage site and human CycD3, amino acids M<sub>1</sub>-L<sub>292</sub> (as in [NCBI/Protein](#) entry NP\_001751.1), N-terminally fused to HIS<sub>6</sub>-Thrombin cleavage site, coexpressed in Sf9 insect cells

**Product identity:** CDK6/CycD3 Lot 004, was confirmed as CDK6/CycD3 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>GST-CDK6</sub>:** 63,279 Da

**Theoretical MW<sub>CycD3</sub>:** 37,260 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

**Activation:** With CAK1

**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

**Storage temperature:** -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.738 µg/µl

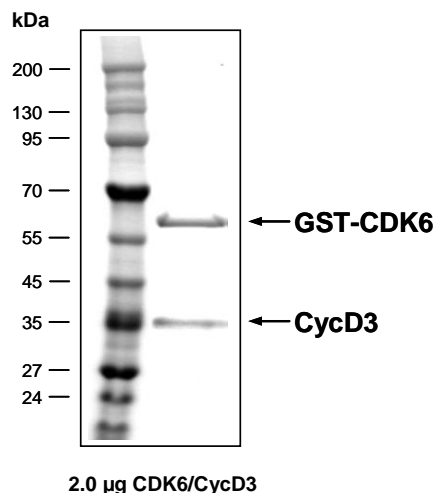
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

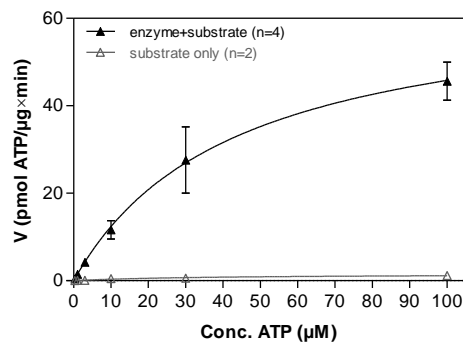
Specific kinase activity (P<sub>i</sub> transfer): 65 pmol/µg × min

ATP-K<sub>M</sub>: 43 µM

**CDK6/CycD3 Lot 004:**  
Coomassie stain



**CDK6/CycD3 Lot 004:**  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



**Determination of K<sub>M</sub> value & Specific activity:**

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: RBER-CHKtide, 80 µg/ml
  - CDK6/CycD3: 2 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

**Additional assay technology:**

CDK6/CycD3 Lot 004 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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## CDK6/CycD3

Product No.: 0051-0373-1

GST-CDK6 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	<b>LVPRGSMEKD</b>	<b>GLCRADQQYE</b>	240
241	<b>CVAEIGEGAY</b>	<b>GKVFKARDLK</b>	<b>NGGRFVALKR</b>	<b>VRVQTGEEGM</b>	<b>PLSTIREVAV</b>	<b>LRHLETFEHP</b>	300
301	<b>NVRLFDVCT</b>	<b>VSRTDRETKL</b>	<b>TLVFEHVDQD</b>	<b>LTTYLDKVPE</b>	<b>PGVPTETIKD</b>	<b>MMFQLLRGLD</b>	360
361	<b>FLHSHRVVHR</b>	<b>DLKPQNILVT</b>	<b>SSGQIKLADF</b>	<b>GLARIYSFQM</b>	<b>ALTSVVVTLW</b>	<b>YRAPEVLLQS</b>	420
421	<b>SYATPVDLWS</b>	<b>VGCIFAEMFR</b>	<b>RKPLFRGSSY</b>	<b>VDQLGKILDV</b>	<b>IGLPGEEDWP</b>	<b>RDVALPRQAF</b>	480
481	<b>HSKSAQPIEK</b>	<b>FVTDIDELGK</b>	<b>DLLLKCLTFN</b>	<b>PAKRISAYSA</b>	<b>LSPYFQDLE</b>	<b>RCKENLDSHL</b>	540
541	<b>PPSQNTSELN</b>	<b>TA</b>					600

1-218: GST **Pink**: Thrombin cleavage site **blue**: CDK6 **boxed**: variation from RefSeq

CDK6 wt <sup>1</sup> Amino Acid Sequence							
1	<b>MEKDGLCRAD</b>	<b>QQYECVAEIG</b>	<b>EGAYGKVFKA</b>	<b>RDLKNGGRFV</b>	<b>ALKRVRVQTG</b>	<b>EEGMPLSTIR</b>	60
61	<b>EVAVLRHLET</b>	<b>FEHPNVVRLF</b>	<b>DVCTVSRTDR</b>	<b>ETKLTLVFEH</b>	<b>VDQDLTTYLD</b>	<b>KVPEPGVPTE</b>	120
121	<b>TIKDMMFQLL</b>	<b>RGLDFLHSHR</b>	<b>VVHRDLKPQN</b>	<b>ILVTSSGQIK</b>	<b>LADFGRLARIY</b>	<b>SFQMALTSVV</b>	180
181	<b>VTLWYRAPEV</b>	<b>LLQSSYATPV</b>	<b>DLWSVGCIFA</b>	<b>EMFRRKPLFR</b>	<b>GSSDVDQLGK</b>	<b>ILDVIGLPGE</b>	240
241	<b>EDWPRDVALP</b>	<b>RQAFHSKSAQ</b>	<b>PIEKFVTDID</b>	<b>ELGKDLLLKC</b>	<b>LTFNPAKRIS</b>	<b>AYSALSHPHYF</b>	300
301	<b>QDLERCKENL</b>	<b>DSHLPPSQNT</b>	<b>SELNTA</b>				360

**blue**: CDK6 sequence expressed in recombinant protein **Red**: variant in recombinant protein

<sup>1</sup>[NCBI/Protein](#) accession number NP\_001250.1

CycD3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPIDPMG <b>HH</b>	<b>HHHH</b> GRRRAS	VAAGI <b>LVPRG</b>	<b>SPGLDGIYAR</b>	GIQAS <b>MELLC</b>	<b>CEGTRHAPRA</b>	60
61	<b>GPDPRLLGDQ</b>	<b>RVLQSLRLLE</b>	<b>ERYVPRASYF</b>	<b>QCQVREIKPH</b>	<b>MRKMLAYWML</b>	<b>EVCEEQRCEE</b>	120
121	<b>EVFPLAMNYL</b>	<b>DRYLSCVPTR</b>	<b>KAQLQLLGAV</b>	<b>CMLLASKLRE</b>	<b>TTPLTIEKLC</b>	<b>IYTDHAVSPR</b>	180
181	<b>QLRDWEVLVL</b>	<b>GKLKWDLAHV</b>	<b>IAHDFLAFIL</b>	<b>HRLSLPRDRQ</b>	<b>ALVKKHAQTF</b>	<b>LALCATDYTF</b>	240
241	<b>AMYPPSMIAT</b>	<b>GSIGAAVQGL</b>	<b>GACSMGDEL</b>	<b>TELLAGITGT</b>	<b>EVDCLRACQE</b>	<b>QIEAALRESL</b>	300
301	<b>REAAQTSSSP</b>	<b>APKAPRGSSS</b>	<b>QGPSQTSTPT</b>	<b>DVTAIHL</b>			360

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycD3 **boxed**: variation from RefSeq

CycD3 wt <sup>2</sup> Amino Acid Sequence							
1	<b>MELLCEGTR</b>	<b>HAPRAGPDPR</b>	<b>LLGDQRLVQS</b>	<b>LLRLEERYVP</b>	<b>RASYFQCVR</b>	<b>EIKPHMRKML</b>	60
61	<b>AYWMLEVCEE</b>	<b>QRCEEVFPL</b>	<b>AMNYLDRLYS</b>	<b>CVPTRKAQLQ</b>	<b>LLGAVCMLLA</b>	<b>SKLRETTPLT</b>	120
121	<b>IEKLCIYTDH</b>	<b>AVSPRQLRDW</b>	<b>EVLVLGKLGK</b>	<b>DLAAVIAHDF</b>	<b>LAFILHRLSL</b>	<b>PRDRQALVKK</b>	180
181	<b>HAQTFLALCA</b>	<b>TDYTFAMYPP</b>	<b>SMIATGSIGA</b>	<b>AVQGLGACSM</b>	<b>SGDELTELLA</b>	<b>GITGTEVDCL</b>	240
241	<b>RACQEQIEAA</b>	<b>LRESLREASQ</b>	<b>TSSSPAPKAP</b>	<b>RGSSSQGPSQ</b>	<b>TSTPTDVTAI</b>	<b>HL</b>	300

**blue**: CycD3 sequence expressed in recombinant protein **Red**: variant in recombinant protein

<sup>2</sup>[NCBI/Protein](#) accession number NP\_001751.1

HGNC identifier: CCND3

S259A: SNP variation see [NCBI/dbSNP](#) ID: rs386700585

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