

Certificate of Analysis



CDK12 wt/CycK

Cyclin-dependent kinase 12

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK12

Synonyms: CRK7, CRKR, CrkRS, CRKRS, hCDK12

Product No.: 1483-1484-1

Lot: 001

Description: Co-expression of human CDK12, amino acids Q₆₉₆-S₁₀₈₂ (as in NCBI/Protein entry NP_057591.2), N-terminal GST-HIS₆ fusion protein with a 3C cleavage site and human CycK, amino acids M₁-S₃₀₀ (as in NCBI/Protein entry NP_001092872.1), N-terminally fused to GST-HIS₆ 3C cleavage site, expressed in Sf9 insect cells

Product identity: CDK12 wt/CycK Lot 001, was confirmed as CDK12/CycK by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-CDK12 wt}: 73,066 Da

Theoretical MW_{CycK}: 63,031 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was activated by coexpression with its physiological cofactor CycK and 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

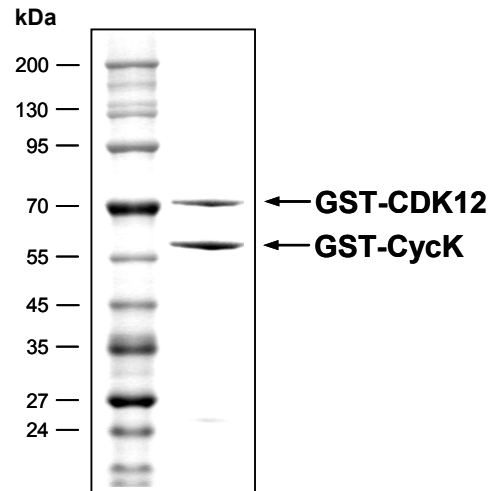
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.763 µg/µl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

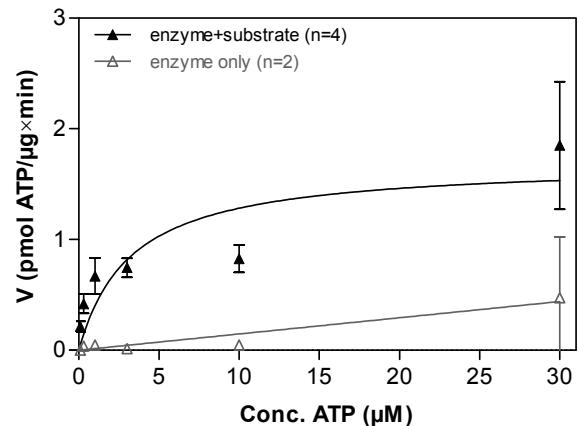
Specific kinase activity (P_i transfer): 1.7 pmol/µg×min
ATP-K_M: 3.2 µM

CDK12 wt/CycK Lot 001: Coomassie stain



2.0 µg CDK12/CycK


CDK12 wt/CycK Lot 001: Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:

- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: RBER-IRStide, 80 µg / ml
 - CDK12 wt/CycK: 2.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: CDK12 wt/CycK Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from 

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CDK12 wt/CycK

Product No.: 1483-1484-1

CDK12 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGQWATF	GGGDHPPKSD	PMG HHHHHG	RDS LEVL FQG	240
241	PQOPYK RPK	ICCP RYGERR	QTESD W GKRC	VDKFD I IGII	GEGTYGQVYK	AKDKDTGELV	300
301	ALKKVR LDNE	KEGFPITAIR	EIKILRQLIH	RSVVMKEIV	TDKQDALDFK	KDKGAFYLVF	360
361	EYMDHDL MGL	LESGLVHFSE	DHIKSFMKQL	MEGLE YCHKK	NFLHRDIKCS	NILLNNSGQI	420
421	KLAD FGLARL	YNSEESR PYT	NKVITLWYRP	PELLLGEERY	TPAIDVWSCG	CILGELFTKK	480
481	PIFQANLELA	QLELISRLCG	SPCPAVWPDV	IKLPYFNTMK	PKKQYRRRLR	EEFSFIPSA A	540
541	LDLLDHMLTL	DPSKRCTAEQ	TLQSDFLKDV	ELSKMAPPDL	PHWQDCHELW	SKKRRRRQRQS	600
601	GVVVEEPPPS	KTSRKETTSG	TSTEPVKNS				660

1-218: GST **Red: HIS6-tag** **Green: 3C** **blue: CDK12 fragment**

CDK12 wt ¹ amino acid sequence							
1	MPNSERHGGK	KDGSGGASGT	LQPSSGGGSS	NSRERHRLVS	KHKRHKSKHS	KDMGLVTPEA	60
61	ASLGTVIKPL	VEYDDISSDS	DTFSDDMAFK	LDRRENDERR	GSDRSDRLHK	HRHHQHRRSR	120
121	DLLKAKQTEK	EKSQEVSSKS	GSMKDRI S GS	SKRSNEETDD	YGKAQVAKSS	SKESRSSKLH	180
181	KEKTRKEREL	KSGHKDRSKS	HRKRETPKSY	KTVDSPKRRS	RSPHRKWSDS	SKQDDSPSGA	240
241	SYGQDYDLSP	SRSHTSSNYD	SYKKSPGSTS	RRQSVSPPYK	EPSAYQSSTR	SPSPYSRRQR	300
301	SVSPYSRRRS	SSYERSGSYS	GRSPSPYGRR	RSSSPFLSKR	SLSRSPLPSR	KSMKRSRSP	360
361	AYSRHSSSHS	KKKRSSSRSR	HSSISPVRLP	LNSSLGAELS	RKKKERAAAA	AAAKMDGKES	420
421	KGSPVFLPRK	ENSSVEAKDS	GLESKKLPRS	VKLEKSAPDT	ELVNVTHLNT	EVKNSSDTGK	480
481	VKLDENSEKH	LVKDLKAQGT	RDSKPIALKE	EIVTPKETET	SEKETPPPLP	TIASPPPLP	540
541	TTTTPPPQTPP	LPPLPIPAL	PQQPPLPPSQ	PAFSQVPASS	TSTLPPSTHS	KTSAVSSQAN	600
601	SQPPVQVSVK	TQVSVTAAIP	HLKTSTLPPL	PLPPLLPGDD	DMDSPKETLP	SKPVKKEKEQ	660
661	RTRHLLTDLP	LPPELPGGDL	SPPDSPEPKA	ITPPQ OPYK	RPKICCPRYG	ERRQTESDWG	720
721	KRCVDKFDII	GIIGEGTYGQ	VYKAKDKDTG	ELVALKKVRL	DNEKEGFPIT	AIREIKILRQ	780
781	LIHRSVVMNK	EIVTDKQDAL	DFKKDKGAFY	LVFEYMDHDL	MGLLESGLVH	FSEDHIKSF M	840
841	QQLMEGLE YC	HKKNFLHRDI	KCSNILLNNS	GQIKLAD FGL	ARLYNSEESR	PYTNKVITLW	900
901	YRPELLLGE	ERYTPAIDVW	SCGCILGELF	TKKPIFQANL	ELAQLELISR	LCGSPCPAVW	960
961	PDVIKLPYFN	TMKPKKQYRR	RLREFFS FIP	SAALDLLDH M	LTLDPSKRCT	AEQTLQSDFL	1020
1021	KDVELSKMAP	PDLPHWQDCH	ELWSKKRRRQ	RQSGVVVEEP	PPSKTSRKET	TSGTSTEPVK	1080
1081	NSSPAPPQPA	PGKVESGAGD	AIGLADITQQ	LNQSELAVLL	NLLQSQTDLS	IPQMAQLLNI	1140
1141	HSNPEMQQQL	EALNQSISAL	TEATSQQQDS	ETMAPEESLK	EAPSAPVILP	SAEQTTLEAS	1200
1201	STPADMQNIL	AVLLSQLMKT	QEPAGSLEEN	NSDKNSGPQG	PRRTPTMPQE	EAAACPPHIL	1260
1261	PPEKRPEPPP	GPPPPPPPPP	LVEGDLS SAP	QELNPAVTAA	LLQLLSQPEA	EPPGHLPEH	1320
1321	QALRPMEYST	RPRPNRTYGN	TDGPETGFSA	IDTDERN SGP	ALTESLVQTL	VKNRTFSGSL	1380
1381	SHLGESSSYQ	GTGSVQFP GD	QDLRFARVPL	ALHPVVGQPF	LKAEGSSNSV	VHAETKLQNY	1440
1441	GELGP GTTGA	SSSGAGLHWG	GPTQSSAYGK	LYRGPTRVPP	RGGRGRGV PY		1500

blue: CDK12 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_057591.2

CycK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHG	RDS LEVLFOG	240
241	PMKENKENS	PSVTSANLDH	TKPCWYWDKK	DLAHTPSQLE	GLDPATEARY	RREGARFIFD	300
301	VGTRLGLHYD	TLATGIIYFH	RFYMFHSFKQ	FPRYVTGACC	LFLAGVEET	PKCKDIIKT	360
361	ARSLNDVQF	QQFGDDPKEE	VMVLERILLQ	TIKFDLQVEH	PYQFLLKYAK	QLKGDKNKIQ	420
421	KLVQMAWTFV	NDSLCTTSL	QWEPEIIAVA	VMYLAGRLCK	FEIQEWTSKP	MYRRWWEQFV	480
481	QDVPVDVLED	ICHQILDLYS	QGKQMPHHT	PHLQPPSL	QPTQVPQVQ	QSQPSQSEEP	540
541	S						600

1-218: GST **Red: HIS6-tag** **Green: 3C** **blue: CycK fragment**

CycK wt ² amino acid sequence							
1	MKENKENS	SVTSANLDHT	KPCWYWDK	LAHTPSQLE	LDPATEARYR	REGARFIFDV	60
61	GTRLGLHYDT	LATGIIYFHR	FYMFHSFKQ	PRYVTGACCL	FLAGKVEETP	KKCKDIIKTA	120
121	RSLNDVQFG	QFGDDPKEEV	MVLERILLQT	IKFDLQVEHP	YQFLLKYAKQ	LKGDKNKIQK	180
181	LVQMAWTFVN	DSLCTTSLQ	WEPEIIAVAV	MYLAGRLCKF	EIQEWTSKPM	YRRWWEQFVQ	240
241	DVPVDVLEDI	CHQILDLYSQ	GKQMPHHTP	HQLQPPSLQ	PTPQVPQVQQ	SQPSQSSEPS	300
301	QPQQKDPQQP	AQQQQPAQQP	KKPSPQPSSP	RQVKRAVVVS	PKEENKAAEP	PPPKIPKIET	360
361	THPPLPPAHP	PPDRKPPLAA	ALGEAEPPGP	VDATDLPKVQ	I PPPAHPAPV	HQPPPLPHRP	420
421	PPPPSSYMT	GMSTTSSYMS	GEGYQSLQSM	MKTEGPSYGA	LPPAYGPPAH	LPYHPHYVYP	480
481	NPPPPVPPP	PASFPPAIP	PPTPGYPPP	PTYNPNFPPP	PPRLPPTHAV	PPHPPGLGL	540
541	PPASYPPPAV	PPGGQPPVPP	PIPPGMPPV	GGLGRAAWMR			600

blue: CycK sequence expressed in fusionprotein

²NCBI/Protein accession number NP_001092872.1

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