

ProQinase™ CDK12 R722C/CycK

cyclin dependent kinase 12

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK12

Synonyms: CRK7, CRKR, CrkRS, CRKRS, hCDK12

Product No.: 1488-1484-1

Lot: 003

Description: Human CDK12, amino acids Q₆₉₆-S₁₀₈₂ (as in [NCBI/Protein](#) entry NP_057591.2) with a R₇₂₂C mutation and human CycK, amino acids M₁-S₃₀₀ (as in [NCBI/Protein](#) entry NP_001092872.1), both N-terminally fused to a GST-HIS₆ sequence followed by a 3C protease cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK12 R722C/CycK Lot 003, was confirmed as CDK12/CycK by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-CDK12 R722C} : 73,012 Da

Theoretical MW_{GST-CycK} : 63,031 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

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: 0.444 µg/µl

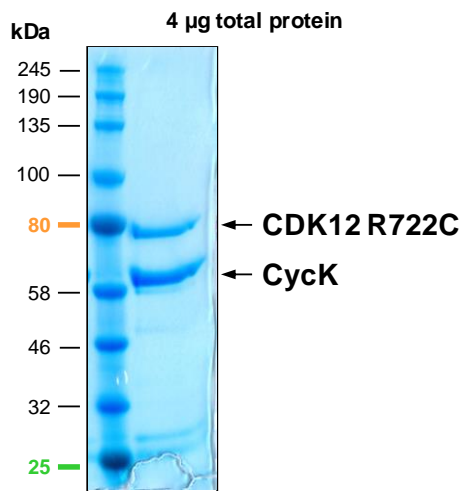
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

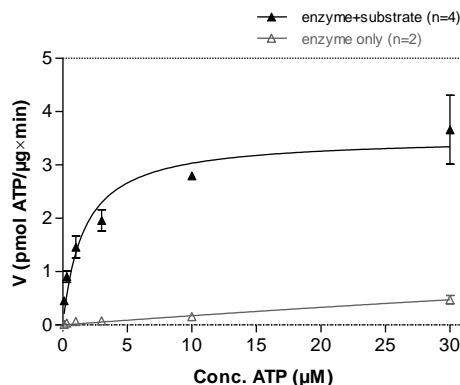
Specific kinase activity (P_i transfer): 3.5 pmol/µg × min

ATP-K_M: 1.6 µM

CDK12 R722C/CycK Lot 003:
Coomassie stain



CDK12 R722C/CycK Lot 003:
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, 40 µg/ml
 - Kinase: 3 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

ProQinase™ CDK12 R722C/CycK

Product No.: 1488-1484-1

GST-CDK12 R722C Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWAQTF	GGGDHPPKSD	PMG HHHHHG	RDS LEVLFGQ	240
241	PQQPYKKRPK	ICCPRYGERR	QTESDWGKC	VDKFDIIGII	GEPTYGQVYK	AKDKDTGELV	300
301	ALKKVRLDNE	KEGFPITAIR	EIKILRQLIH	RSVNMKEIV	TDKQDALDFK	KDKGAFYLVF	360
361	EYMDHDLMGL	LESGLVHFSE	DHIKSFMKQL	MEGLEYPCHK	NFLHRDIKCS	NILLNNSGQI	420
421	KLADFGLARL	YNSEESRPT	NKVITLWYRP	PELLLGEERY	TPAIDVWSCG	CILGELFTKK	480
481	PIFQANLELA	QLELISRLCG	SPCPAVWPDV	IKLPYFNTMK	PKKQYRRRLR	EEFSFIPSA	540
541	LDLLDHMLTL	DPSKRCTAEQ	TLQSDFLKDV	ELSKMAPDDL	PHWQDCHELW	SKKRRRQRQS	600
601	GVVVEEPPPS	KTSRKETTS	TSTEPVKNS				660

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CDK12 fragment **boxed**: R722C mutation

CDK12 wt ¹ Amino Acid Sequence							
1	MPNSERHGGK	KDGS GGASGT	LQPSSGGGSS	NSRERHRLVS	KHKRHKSKHS	KDMGLVTPEA	60
61	ASLGTVIKPL	VEYDDISSDS	DTFSDDMAFK	LDRRENDERR	GSDRSDDLHK	HRHHQHRRSR	120
121	DLLKAKQTEK	EKSQEVSSKS	GSMKDRIKGS	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	GLESKLLPRS	VKLEKSAPDT	ELVNVTHLNT	EVKNSSDTGK	480
481	VKLDENSEKH	LVKDLKAQGT	RDSKPIALKE	EIVTPKETET	SEKETPPPLP	TIASPPPLP	540
541	TTTPPPQTPP	LPPLPPIPAL	PQQPPLPPSQ	PAFSQVPASS	TSTLPPSTHS	KTSAVSSQAN	600
601	SQPPVQVSVK	TQVSVTAAIP	HLKTSTLPP	PLPPLPGDD	DMDSPKETLP	SKPVKKEKEQ	660
661	RTRHLLTDL	LPPELPGGDL	SPPDSPEPKA	ITPPQ QPYK	RPKICCPRYG	ERRQTESDWG	720
721	KRCVDKFDII	GIIGEGTYGQ	VYKAKDKTG	ELVALKKVRL	DNEKEGFPIT	AIREIKILRQ	780
781	LIHRSVVNMK	EIVTDKQDAL	DFKKDKGAFY	LVFEYMDHDL	MGLLESGLVH	FSEDHIKSEF	840
841	QQLMEGLEYC	HKKNFLHRDI	KCSNILLNNS	GQIKLADFGL	ARLYNSEESR	PYTNKVITLW	900
901	YRPELLLGE	ERYTPAIDVW	SCGCILGELF	TKKPIFQANL	ELAQLELISR	LCGSPCPAVW	960
961	PDVIKLPYFN	TMKPKKQYRR	RLREEFSFIP	SAALDLLDHM	LTLDPSKRCT	AEQTLQSDFL	1020
1021	KDVELSKMAP	PDLPHWQDCH	ELWSKRRRQ	RQSGVVVEEP	PPSKTSRKET	TSGTSTEPVK	1080
1081	NSSPAPPQPA	PGKVESGAGD	AIGLADITQQ	LNQSELAVLL	NLLQSQTDL	IPQMAQLLNI	1140
1141	HSNPEMQQL	EALNQSISAL	TEATSQQQDS	ETMAPEESLK	EAPSAPVILP	SAEQTTLEAS	1200
1201	STPADMQNIL	AVLLSQLMKT	QEPAGSLEEN	NSDKNSGPQG	PRRTPTMPQE	EAAACPPHIL	1260
1261	PPEKRPPEPP	GPPPPPPPPP	LVEGDLSSAP	QELNPAVTAA	LLQLLSQPEA	EPPGHLPEH	1320
1321	QALRPMEYST	RPRPNRTYGN	TDGPETGFS	IDTDERNNGP	ALTESLVQTL	VKNRTFSGSL	1380
1381	SHLGESSSYQ	GTGSVQFPD	QDLRFARVPL	ALHPVVGQPF	LKAEGSSNSV	VHAETKLQNY	1440
1441	GELGPGTTGA	SSSGAGLHWG	GPTQSSAYGK	LYRGPTRVPP	RGGRGRGVPP		1500

blue: CDK12 sequence expressed in recombinant protein **Red**: variant in recombinant protein

¹[NCBI/Protein](https://www.ncbi.nlm.nih.gov/Protein/np_057591.2) accession number NP_057591.2

GST-CycK Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFG	240
241	MKENKENS	PSVTANLDH	TKPCWYDCK	DLAHTPSQLE	GLDPATEARY	RREGARFID	300
301	VGTRLGLHYD	TLATGIIYFH	RFYMFHSFKQ	FPRYVTGACC	LFLAGKVEET	PKKCKDIKT	360
361	ARSLNDVQF	QFGDDPKEE	VMVLERILLQ	TIKFDLQVEH	PYQFLLKYAK	QLKGDKNKIQ	420
421	KLVQMAWTFV	NDSLCTTSL	QWEPEIIAVA	VMYLAGRLCK	FEIQEWTSKP	MYRRWWEQFV	480
481	QDVPVDVLED	ICHQILDLYS	QGKQMPHHT	PHLQPPSL	QPTPQVPQVQ	QSQPSQSSEP	540
541	S						600

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

CycK wt² Amino Acid Sequence

1	MKENKENS	SVTSANLDHT	KPCWYDCKD	LAHTPSQLE	LDPATEARYR	REGARFIDV	60
61	GTRLGLHYDT	LATGIIYFHR	FYMFHSFKQF	PRYVTGACCL	FLAGKVEETP	KKCKDIIKTA	120
121	RSLNDVQFG	QFGDDPKEEV	MVLERILLQT	IKFDLQVEHP	YQFLLKYAQ	LKGDKNKIQK	180
181	LVQMAWTFVN	DSLCTTSLQ	WEPEIIAVAV	MYLAGRLCKF	EIQEWTSKPM	YRRWWEQFVQ	240
241	DVPVDVLEDI	CHQILDLYSQ	GKQMPHHTP	HQLQPPSLQ	PTPQVPQVQ	SQPSQSSEPS	300
301	QPQQKDPQQP	AQQQQPAQQP	KKPSPQSSP	RQVKRAVVVS	PKEENKAAEP	PPPKIPKIET	360
361	THPPLPPAHP	PPDRKPPLAA	ALGEAEPGP	VDATDLPKVQ	IPPPAHPAPV	HQPPPLPHRP	420
421	PPPPSSYMT	GMSTTSSYMS	GEGYQSLQSM	MKTEGPSYGA	LPPAYGPPAH	LPYHPHYVPP	480
481	NPPPPVPPP	PASFPAPAIP	PPTPGYPPP	PTYNPNFPPP	PPRLPPTHAV	PPHPPGLGL	540
541	PPASYPPPAV	PPGGQPPVPP	PIPPPGMPPV	GGLGRAAWMR			600

blue: CycK sequence expressed in recombinant protein

²NCBI/Protein accession number NP_001092872.1