

## 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: 001

**Description:** Human CDK12, amino acids Q<sub>696</sub>-S<sub>1082</sub> (as in [NCBI/Protein](#) entry NP\_057591.2) with a R<sub>722</sub>C mutation and human CycK, amino acids M<sub>1</sub>-S<sub>300</sub> (as in [NCBI/Protein](#) entry NP\_001092872.1), both N-terminally fused to a GST-HIS<sub>6</sub> sequence followed by a 3C protease cleavage site, coexpressed in Sf9 insect cells

**Product identity:** CDK12 R722C/CycK Lot 001, was confirmed as CDK12/CycK by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>GST-CDK12 R722C</sub> : 73,012 Da

**Theoretical MW**<sub>GST-CycK</sub> : 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:** 0.814 µg/µl

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

**Biochemical Parameters:**

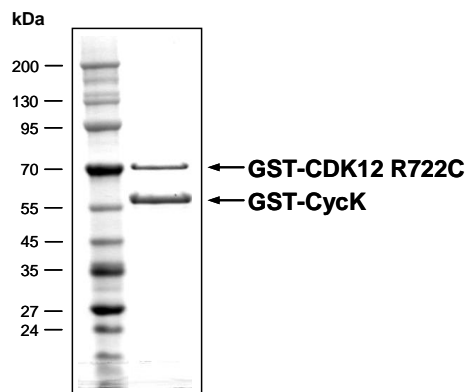
Specific kinase activity (P<sub>i</sub> transfer): 1.4 pmol/µg × min

ATP-K<sub>M</sub>: 0.7 µM

**Additional assay technology:**

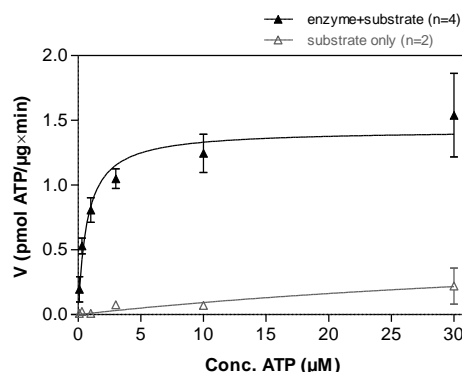
CDK12 R722C/CycK Lot 001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

### CDK12 R722C/CycK Lot 001: Coomassie stain



2.0 µg CDK12 R722C/CycK

### CDK12 R722C/CycK Lot 001: 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-IRStide, 80 µg/ml
  - Kinase: 2 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

# 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	GDVTKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWAQTF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PQQPYKKRPK	ICCPRYGERR	QTESDWGKCC	VDFKDIIGII	GEPTYGQVYK	AKDKDTGELV	300
301	ALKKVRLDNE	KEGFPITAIR	EIKILRQLIH	RSVVMKEIV	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	KTSRKETTSG	TSTEPVKNS				660

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

CDK12 wt <sup>1</sup> Amino Acid Sequence							
1	MPNSERHGGK	KDGS GGASGT	LQPSSGGGSS	NSRERHRLVS	KHKRHKSKHS	KDMGLVTPEA	60
61	ASLGTVIKPL	VEYDDISSDS	DTFSDDMAFK	LDRRENDERR	GSDRSDDLHK	HRHHQHRRSR	120
121	DLLKAKQTEK	EKSQEVSSKS	GSMKDRISSG	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	KSMKRSRSRP	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	TTTPPPQTPP	LPPLPPIPAL	PQQPPLPPSQ	PAFSQVPASS	TSTLPPSTHS	KTSAVSSQAN	600
601	SQPPVQVSVK	TQVSVTAAIP	HLKTSTLPP	PLPPLPGDD	DMDSPKETLP	SKPVKKEKEQ	660
661	RTRHLLTDLP	LPPELPGGDL	SPPDSPEPKA	ITPPQQPYKK	RPKICCPRYG	ERRQTESDWG	720
721	KRCVDKFDII	GIIGEGTYGQ	VYKAKDKDTG	ELVALKKVRL	DNEKEGFPI	AIREIKILRQ	780
781	LIHRSVVNMK	EIVTDKQDAL	DFKKDKGAFY	LVFEYMDHDL	MGLLESGLVH	FSEDIKSEFM	840
841	KQLMEGLEYC	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	EPPGHLPEHE	1320
1321	QALRPMEYST	RPRPNRTYGN	TDGPETGFS	IDTDERNRSGP	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

<sup>1</sup>NCBI/Protein accession number NP\_057591.2

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**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	PSVTSANLDH	TKPCWYWDKK	DLAHTPSQLE	GLDPATEARY	RREGARFIFD	300
301	VGTRLGLHYD	TLATGIIYFH	RFYMFHSFKQ	FPRYVTGACC	LFLAGKVEET	PKKCKDIKT	360
361	ARSLNDVQF	QFGDDPKEE	VMVLERILLQ	TIKFDLQVEH	PYQFLLYAK	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 wt<sup>2</sup> Amino Acid Sequence**

1	MKENKENS	SVTSANLDHT	KPCWYWDKKD	LAHTPSQLE	LDPATEARYR	REGARFIFDV	60
61	GTRLGLHYDT	LATGIIYFHR	FYMFHSFKQF	PRYVTGACCL	FLAGKVEETP	KKCKDIKTA	120
121	RSLNDVQFG	QFGDDPKEEV	MVLERILLQT	IKFDLQVEHP	YQFLLYAKQ	LKGDKNKIQK	180
181	LVQMAWTFVN	DSLCTTSLQ	WEPEIIAVAV	MYLAGRLCKF	EIQEWTSKPM	YRRWWEQFVQ	240
241	DVPVDVLEDI	CHQILDLYSQ	GKQMPHHTP	HQLQPPSLQ	PTPQVPQVQQ	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	PPGQPPVPP	PIPPGMPPV	GGLGRAAWMR			600

blue: CycK sequence expressed in recombinant protein

<sup>2</sup>NCBI/Protein accession number NP\_001092872.1

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