Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase$^{\text{TM}}$ CDK12/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: 013

Description: 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, co-expressed in Sf9 insect cells

Product identity: CDK12/CycK Lot 013, was confirmed as CDK12/CycK by mass spectroscopy LC-ESI-MS/MS

Theoretical $\text{MW}_{\text{GST-CDK12}}$: 73,066 Da
Theoretical $\text{MW}_{\text{GST-CycK}}$: 63,031 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With CAK

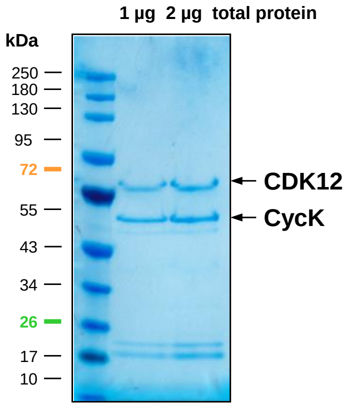
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

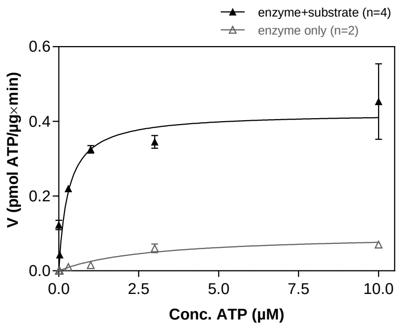
Protein concentration: 0.244 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 0.4 pmol/µg x min
ATP-K_M: 0.3 µM

CDK12/CycK Lot 013:
Coomassie stain



CDK12/CycK Lot 013:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: RBER-IRStide 80 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Sequence information

GST-CDK12 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	KRIEAIPQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG**HHHHHH**G	RDS**LEVLFQG**	240
241	**P**Q**QPYKKRPK**	**ICCPRYGERR**	**QTESDWGKRC**	**VDKFDIIGII**	**GEGTYGQVYK**	**AKDKDTGELV**	300
301	**ALKKVRLDNE**	**KEGFPITAIR**	**EIKILRQLIH**	**RSVVNMKEIV**	**TDKQDALDFK**	**KDKGAFYLVF**	360
361	**EYMDHDLMGL**	**LESGLVHFSE**	**DHIKSFMKQL**	**MEGLEYCHKK**	**NFLHRDIKCS**	**NILLNNSGQI**	420
421	**KLADFGLARL**	**YNSEESRPYT**	**NKVITLWYRP**	**PELLLGEERY**	**TPAIDVWSCG**	**CILGELFTKK**	480
481	**PIFQANLELA**	**QLELISRLCG**	**SPCPAVWPDV**	**IKLPYFNTMK**	**PKKQYRRRLR**	**EEFSFIPSAA**	540
541	**LDLLDHMLTL**	**DPSKRCTAEQ**	**TLQSDFLKDV**	**ELSKMAPPDL**	**PHWQDCHELW**	**SKKRRRQRQS**	600
601	**GVVVEEPPPS**	**KTSRKETTSG**	**TSTEPVKNS**				660

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **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	GSMKDRISGS	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	KSMKSRSRSP	360
361	AYSRHSSSHS	KKKRSSSRSR	HSSISPVRLP	LNSSLGAELS	RKKKERAAAA	AAAKMDGKES	420
421	KGSPVFLPRK	ENSSVEAKDS	GLESKKLPRS	VKLEKSAPDT	ELVNVTHLNT	EVKNSSDTGK	480
481	VKLDENSEKH	LVKDLKAQGT	RDSKPIALKE	EIVTPKETET	SEKETPPPLP	TIASPPPPLP	540
541	TTTPPPQTPP	LPPLPPIPAL	PQQPPLPPSQ	PAFSQVPASS	TSTLPPSTHS	KTSAVSSQAN	600
601	SQPPVQVSVK	TQVSVTAAIP	HLKTSTLPPL	PLPPLLPGDD	DMDSPKETLP	SKPVKKEKEQ	660
661	RTRHLLTDLP	LPPELPGGDL	SPPDSPEPKA	ITPPQ**QPYKK**	**RPKICCPRYG**	**ERRQTESDWG**	720
721	**KRCVDKFDII**	**GIIGEGTYGQ**	**VYKAKDKDTG**	**ELVALKKVRL**	**DNEKEGFPIT**	**AIREIKILRQ**	780
781	**LIHRSVVNMK**	**EIVTDKQDAL**	**DFKKDKGAFY**	**LVFEYMDHDL**	**MGLLESGLVH**	**FSEDHIKSFM**	840
841	**KQLMEGLEYC**	**HKKNFLHRDI**	**KCSNILLNNS**	**GQIKLADFGL**	**ARLYNSEESR**	**PYTNKVITLW**	900
901	**YRPPELLLGE**	**ERYTPAIDVW**	**SCGCILGELF**	**TKKPIFQANL**	**ELAQLELISR**	**LCGSPCPAVW**	960
961	**PDVIKLPYFN**	**TMKPKKQYRR**	**RLREEFSFIP**	**SAALDLLDHM**	**LTLDPSKRCT**	**AEQTLQSDFL**	1020
1021	**KDVELSKMAP**	**PDLPHWQDCH**	**ELWSKKRRRQ**	**RQSGVVVEEP**	**PPSKTSRKET**	**TSGTSTEPVK**	1080
1081	**NS**SPAPPQPA	PGKVESGAGD	AIGLADITQQ	LNQSELAVLL	NLLQSQTDLS	IPQMAQLLNI	1140
1141	HSNPEMQQQL	EALNQSISAL	TEATSQQQDS	ETMAPEESLK	EAPSAPVILP	SAEQTTLEAS	1200
1201	STPADMQNIL	AVLLSQLMKT	QEPAGSLEEN	NSDKNSGPQG	PRRTPTMPQE	EAAACPPHIL	1260
1261	PPEKRPPEPP	GPPPPPPPPP	LVEGDLSSAP	QELNPAVTAA	LLQLLSQPEA	EPPGHLPHEH	1320
1321	QALRPMEYST	RPRPNRTYGN	TDGPETGFSA	IDTDERNSGP	ALTESLVQTL	VKNRTFSGSL	1380
1381	SHLGESSSYQ	GTGSVQFPGD	QDLRFARVPL	ALHPVVGQPF	LKAEGSSNSV	VHAETKLQNY	1440
1441	GELGPGTTGA	SSSGAGLHWG	GPTQSSAYGK	LYRGPTRVPP	RGGRGRGVPY		1500

blue: CDK12 sequence expressed in recombinant protein

[1] NCBI/Protein accession number NP_057591.2

GST-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	KRIEAIPQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG**HHHHHH**G	RDS**LEVLFQG**	240
241	**PMKENKENSS**	**PSVTSANLDH**	**TKPCWYWDKK**	**DLAHTPSQLE**	**GLDPATEARY**	**RREGARFIFD**	300
301	**VGTRLGLHYD**	**TLATGIIYFH**	**RFYMFHSFKQ**	**FPRYVTGACC**	**LFLAGKVEET**	**PKKCKDIIKT**	360
361	**ARSLLNDVQF**	**GQFGDDPKEE**	**VMVLERILLQ**	**TIKFDLQVEH**	**PYQFLLKYAK**	**QLKGDKNKIQ**	420
421	**KLVQMAWTFV**	**NDSLCTTLSL**	**QWEPEIIAVA**	**VMYLAGRLCK**	**FEIQEWTSKP**	**MYRRWWEQFV**	480
481	**QDVPVDVLED**	**ICHQILDLYS**	**QGKQQMPHHT**	**PHQLQQPPSL**	**QPTPQVPQVQ**	**QSQPSQSSEP**	540
541	**S**						600

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

CycK wt[2] Amino Acid Sequence

1	**MKENKENSSP**	**SVTSANLDHT**	**KPCWYWDKKD**	**LAHTPSQLEG**	**LDPATEARYR**	**REGARFIFDV**	60
61	**GTRLGLHYDT**	**LATGIIYFHR**	**FYMFHSFKQF**	**PRYVTGACCL**	**FLAGKVEETP**	**KKCKDIIKTA**	120
121	**RSLLNDVQFG**	**QFGDDPKEEV**	**MVLERILLQT**	**IKFDLQVEHP**	**YQFLLKYAKQ**	**LKGDKNKIQK**	180
181	**LVQMAWTFVN**	**DSLCTTLSLQ**	**WEPEIIAVAV**	**MYLAGRLCKF**	**EIQEWTSKPM**	**YRRWWEQFVQ**	240
241	**DVPVDVLEDI**	**CHQILDLYSQ**	**GKQQMPHHTP**	**HQLQQPPSLQ**	**PTPQVPQVQQ**	**SQPSQSSEPS**	300
301	QPQQKDPQQP	AQQQQPAQQP	KKPSPQPSSP	RQVKRAVVVS	PKEENKAAEP	PPPKIPKIET	360
361	THPPLPPAHP	PPDRKPPLAA	ALGEAEPPGP	VDATDLPKVQ	IPPPAHPAPV	HQPPPLPHRP	420
421	PPPPPSSYMT	GMSTTSSYMS	GEGYQSLQSM	MKTEGPSYGA	LPPAYGPPAH	LPYHPHVYPP	480
481	NPPPPPVPPP	PASFPPPAIP	PPTPGYPPPP	PTYNPNFPPP	PPRLPPTHAV	PPHPPPGLGL	540
541	PPASYPPPAV	PPGGQPPVPP	PIPPPGMPPV	GGLGRAAWMR			600

blue: CycK sequence expressed in recombinant protein

[2] NCBI/Protein accession number NP_001092872.1
HGNC identifier CycK: CCNK