Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ CDK9/CycK

Cyclin dependent kinase 9 / CyclinK

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK9

Synonyms: C-2k; CDC2L4; PITALRE; TAK; CDC2-related kinase

Product No.: 0371-1484-1

Lot: 004

Description: Coexpression of human CDK9, full length, amino acids M_1-F_{372} (as in NCBI/Protein entry NP_001252.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, and human human CycK, amino acids M_1-S_{300} (as in NCBI/Protein entry NP_001092872.1), N-terminally fused to GST-HIS_6 3C cleavage site, both expressed in Sf9 insect cells

Product identity: CDK9/CycK Lot 004, was confirmed as CDK9/CycK by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK9}$: 72,173 Da
Theoretical $MW_{GST\text{-}CycK}$: 63,031 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

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

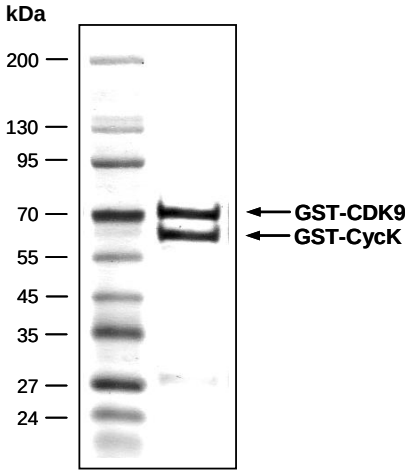
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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

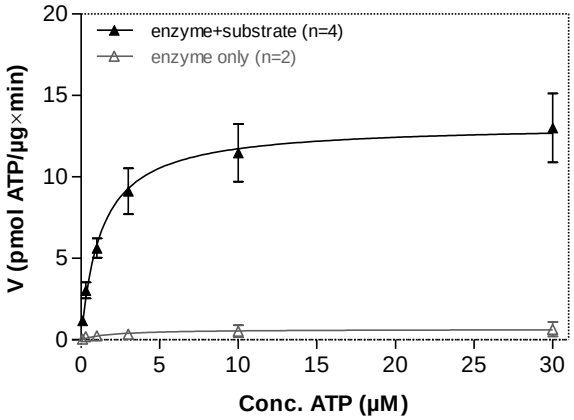
Biochemical Parameters:
Specific kinase activity (P_i transfer): 13 pmol/µg×min
ATP-K_M: 1.3 µM

CDK9/CycK Lot 004:
Coomassie stain



2.0 µg CDK9/CycK

CDK9/CycK Lot 004:
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 $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg / ml $PEG_{20.000}$
  ATP (variable)
  Substrate: RBER-CHKtide, 80 µg/ml
  CDK9/CycK: 2.0 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology: CDK9/CycK Lot 004 was also successfully tested by Reaction Biology 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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ProQinase™ CDK9/CycK

Product No.: 0371-1484-1

Recombinant Proteins

CDK9 Recombinant Fusion Protein Amino Acid Sequence

```
1    MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID 60
61   GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241  LVPRGSPGLD GICSRMAKQY DSVECPFCDE VSKYEKLAKI GQGTFGEVFK ARHRKTGQKV 300
301  ALKKVLMENE KEGFPITALR EIKILQLLKH ENVVNLIEIC RTKASPYNRC KGSIYLVFDF 360
361  CEHDLAGLLS NVLVKFTLSE IKRVMQMLLN GLYYIHRNKI LHRDMKAANV LITRDGVLKL 420
421  ADFGLARAFS LAKNSQPNRY TNRVVTLWYR PPELLLGERD YGPPIDLWGA GCIMAEMWTR 480
481  SPIMQGNTEQ HQLALISQLC GSITPEVWPN VDNYELYEKL ELVKGQKRKV KDRLKAYVRD 540
541  PYALDLIDKL LVLDPAQRID SDDALNHDFF WSDPMPSDLK GMLSTHLTSM FEYLAPPRRK 600
601  GSQITQQSTN QSRNPATTNQ TEFERVF                                     660
```

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin protease cleavage site **blue**: CDK9

CDK9 wt[1] amino acid sequence

```
  1  MAKQYDSVEC PFCDEVSKYE KLAKIGQGTF GEVFKARHRK TGQKVALKKV LMENEKEGFP 60
 61  ITALREIKIL QLLKHENVVN LIEICRTKAS PYNRCKGSIY LVFDFCEHDL AGLLSNVLVK 120
121  FTLSEIKRVM QMLLNGLYYI HRNKILHRDM KAANVLITRD GVLKLADFGL ARAFSLAKNS 180
181  QPNRYTNRVV TLWYRPPELL LGERDYGPPI DLWGAGCIMA EMWTRSPIMQ GNTEQHQLAL 240
241  ISQLCGSITP EVWPNVDNYE LYEKLELVKG QKRKVKDRLK AYVRDPYALD LIDKLLVLDP 300
301  AQRIDSDDAL NHDFFWSDPM PSDLKGMLST HLTSMFEYLA PPRRKGSQIT QQSTNQSRNP 360
361  ATTNQTEFER VF                                                    420
```

blue: CDK9 sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_001252.1

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 PMGHHHHHHG RDSLEVLFQG 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
```

Red: HIS6-tag **Green:** 3C protease 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 fusionprotein

[2]NCBI/Protein accession number NP_001092872.1