

Certificate of Analysis



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 M₁-F₃₇₂ (as in NCBI/Protein entry NP_001252.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, and human human CycK, amino acids M₁-S₃₀₀ (as in NCBI/Protein entry NP_001092872.1), N-terminally fused to GST-HIS₆ 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-CDK9}: 72,173 Da

Theoretical MW_{GST-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

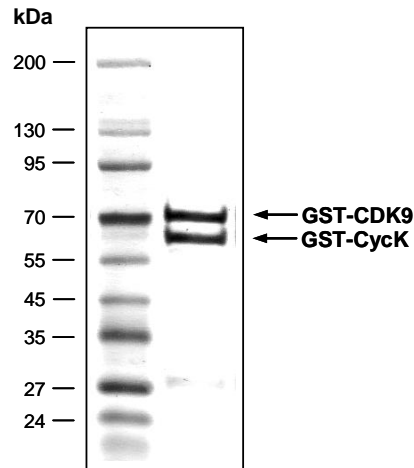
ATP-K_M: 1.3 µM

Additional assay technology: CDK9/CycK Lot 004

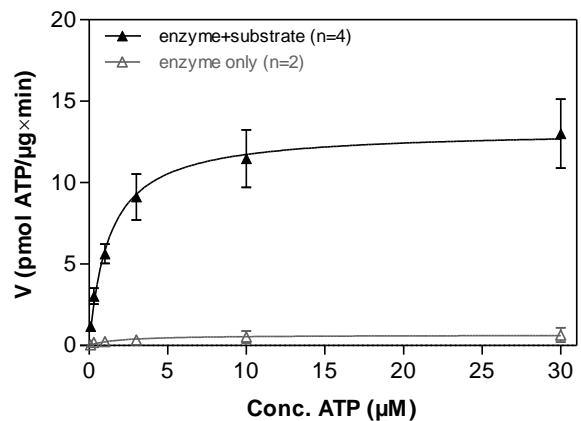
was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



**CDK9/CycK Lot 004:
Coomassie stain**



**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 MgCl₂
 - 3 mM MnCl₂
 - 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)

CDK9/CycK

Product No.: 0371-1484-1

CDK9 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYYYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRMAKQY	DSVECPFCDE	VSKYEKLAKI	GQGTFGGEVFK	ARHRKTGQKV	300
301	ALKKVLMEVE	KEGFPITALR	EIKILQLLKH	ENVVNLIEIC	RTKASPYNRC	KGSIYLVFDF	360
361	CEHDLAGLLS	NVLVKFTLSE	IKRVMQMLLN	GLYYIHRNKI	LHRDMKAANV	LITRDGVLKL	420
421	ADFGLARAFS	LAKNSQPNRY	TNRVVTWYR	PELLLGERD	YGPPIDLWGA	GCIMAEMWTR	480
481	SPIMQGNTEQ	HQLALISQLC	GSITPEVWPN	VDNYELYEKL	ELVKGQKRKV	KDRLKAYVRD	540
541	PYALDLIDKL	LVLDPAQRID	SDDALNHDFE	WSDPMPSDLK	GMLSTHLSM	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	KLAKIGQGTG	GEVFKARHRK	TGQKVALKKV	LMENEKEGFP	60
61	ITALREIKIL	QLLKHENVVN	LIEICRTKAS	PYNRCKGSIY	LVFDFCEHDL	AGLLSNVLVK	120
121	FTLSEIKRVM	QMLLNGLYYI	HRNKILHRDM	KAANVLITRD	GVLKLADFGF	ARAFSLAKNS	180
181	QPNRYTNRV	TLWYRPELL	LGERDYGPPI	DLWGAGCIMA	EMWTRSPIMQ	GNTAQHQLAL	240
241	ISQLCGSITP	EVWPNVDNVE	LYEKLELVKG	QKRKVKDRLK	AYVRDPYALD	LIDKLLVLDP	300
301	AQRIDSDDAL	NHDFWSDPM	PSDLKGLMST	HLTSMFEYLA	PPRRKGSQIT	QQSTNQSRNP	360
361	ATTNQTEFER	VF					420

blue: CDK9 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_001252.1

CycK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYYYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RDSLEVLVQ	240
241	PMKENKENS	PSVTSANLDH	TKPCWYWDKK	DLAHTPSQLE	GLDPATEARY	RREGARFIFD	300
301	VGTRLGLHYD	TLATGIIYFH	RFYMFHSFKQ	FPRYVTGACC	LFLAGKVEET	PKCKDIIKT	360
361	ARSLNDVQF	QFGDDPKEE	VMVLERILLQ	TIKFDLQVEH	PYQFLLKYAK	QLKGDKNKIQ	420
421	KLVQMAWTFV	NDSLCTTSL	QWEPEIIAVA	VMYLAGRLCK	FEIQEWTSKP	MYRRWWEQFV	480
481	QDVPVDVLED	ICHQILDLYS	QKQMPHHT	PHQLQPPSL	QPTPQVPQVQ	QSQPSQSEPS	540
541	S						600

Red: HIS6-tag Green: 3C protease cleavage site blue: CycK fragment

CycK wt ² amino acid sequence							
1	MKENKENS	SVTSANLDHT	KPCWYWDK	LAHTPSQLEG	LDPATEARYR	REGARFIFDV	60
61	GTRLGLHYDT	LATGIIYFHR	FYMFHSFKQF	PRYVTGACCL	FLAGKVEETP	KKCKDIIKTA	120
121	RSLNDVQFG	QFGDDPKEE	MVLERILLQ	IKFDLQVEHP	YQFLLKYAKQ	LKGDKNKIQK	180
181	LVQMAWTFVN	DSLCTTSLQ	WEPEIIAVAV	MYLAGRLCKF	EIQEWTSKPM	YRRWWEQFVQ	240
241	DVPVDVLEDI	CHQILDLYS	GKQMPHHT	HQLQPPSLQ	PTPQVPQVQ	SQPSQSEPS	300
301	QPQQKDPQQP	AQQQPAQQP	KKPSPQSSP	RQVKRAVVVS	PKEENKAAEP	PPPKIPKIET	360
361	THPPLPAHP	PPDRKPPLAA	ALGEAEPGP	VDATDLPKVQ	IPPAHPAPV	HQPPPLPHRP	420
421	PPPPSSYMT	GMSTSSYMS	GEGYQSLQSM	MKTEGPSYGA	LPPAYGPPAH	LPYHPHYVPP	480
481	NPPPPVPPP	PASFPAPAIP	PPTPGYPPP	PTYNPNFPPP	PPRLPPTHAV	PPHPPGLGL	540
541	PPASYPVAV	PPGGQPPVPP	PIPPPGMPPV	GGLGRAAWMR			600

blue: CycK sequence expressed in fusionprotein

²NCBI/Protein accession number NP_001092872.1

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