Certificate of Analysis

REACTION BIOLOGY

ProQinase™ CDK9/CycT1

cyclin dependent kinase 9 / CyclinT1

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK9

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

Product No.: 0371-0345-1

Lot: 014

Description: 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 CycT1, full length, amino acids M_1-K_{726} (as in NCBI/Protein entry NP_001231.2), N-terminal HIS_6 fusion protein with a Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK9/CycT1 Lot 014, has been verified by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK9}$: 72,173 Da
Theoretical $MW_{HIS\text{-}CycT1}$: 85,441 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

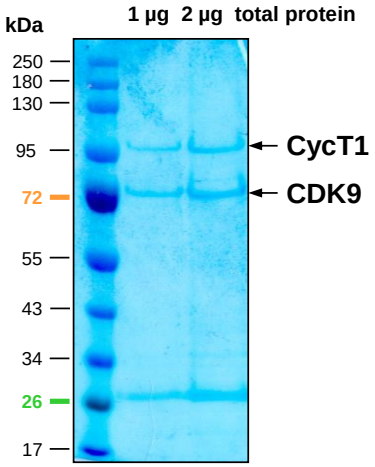
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

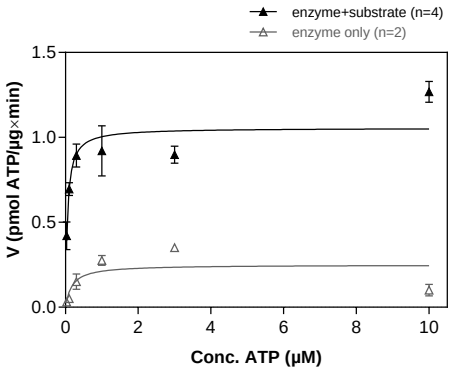
Protein concentration: 0.102 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 1.1 pmol/µg x min
ATP-K_M: 0.05 µM

CDK9/CycT1 Lot 014:
Coomassie stain



CDK9/CycT1 Lot 014:
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-CHKtide 80 µg/ml
  Kinase: 0.5 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Recombinant Proteins

Sequence information

GST-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 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 recombinant protein

[1]NCBI/Protein accession number NP_001252.1

Recombinant Proteins

HIS-CycT1 Recombinant Fusion Protein Amino Acid Sequence

1	MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGIYAR GIQASMEGER KNNNKRWYFT	60
61	REQLENSPSR RFGVDPDKEL SYRQQAANLL QDMGQRLNVS QLTINTAIVY MHRFYMIQSF	120
121	TQFPGNSVAP AALFLAAKVE EQPKKLEHVI KVAHTCLHPQ ESLPDTRSEA YLQQVQDLVI	180
181	LESIILQTLG FELTIDHPHT HVVKCTQLVR ASKDLAQTSY FMATNSLHLT TFSLQYTPPV	240
241	VACVCIHLAC KWSNWEIPVS TDGKHWWEYV DATVTLELLD ELTHEFLQIL EKTPNRLKRI	300
301	WNWRACEAAK KTKADDRGTD EKTSEQTILN MISQSSSDTT IAGLMSMSTS TTSAVPSLPV	360
361	SEESSSNLTS VEMLPGKRWL SSQPSFKLEP TQGHRTSENL ALTGVDHSLP QDGSNAFISQ	420
421	KQNSKSVPSA KVSLKEYRAK HAEELAAQKR QLENMEANVK SQYAYAAQNL LSHHDSHSSV	480
481	ILKMPIEGSE NPERPFLEKA DKTALKMRIP VAGGDKAASS KPEEIKMRIK VHAAADKHNS	540
541	VEDSVTKSRE HKEKHKTHPS NHHHHHNHHS HKHSHSQLPV GTGNKRPGDP KHSSQTSNLA	600
601	HKTYSLSSSF SSSSSTRKRG PSEETGGAVF DHPAKIAKST KSSSLNFSFP SLPTMGQMPG	660
661	HSSDTSGLSF SQPSCKTRVP HSKLDKGPTG ANGHNTTQTI DYQDTVNMLH SLLSAQGVQP	720
721	TQPTAFEFVR PYSDYLNPRS GGISSRSGNT DKPRPPPLPS EPPPPLPPLP K	780

Red: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycT1

CycT1 wt[2] Amino Acid Sequence

1	MEGERKNNNK RWYFTREQLE NSPSRRFGVD PDKELSYRQQ AANLLQDMGQ RLNVSQLTIN	60
61	TAIVYMHRFY MIQSFTQFPG NSVAPAALFL AAKVEEQPKK LEHVIKVAHT CLHPQESLPD	120
121	TRSEAYLQQV QDLVILESII LQTLGFELTI DHPHTHVVKC TQLVRASKDL AQTSYFMATN	180
181	SLHLTTFSLQ YTPPVVACVC IHLACKWSNW EIPVSTDGKH WWEYVDATVT LELLDELTHE	240
241	FLQILEKTPN RLKRIWNWRA CEAAKKTKAD DRGTDEKTSE QTILNMISQS SSDTTIAGLM	300
301	SMSTSTTSAV PSLPVSEESS SNLTSVEMLP GKRWLSSQPS FKLEPTQGHR TSENLALTGV	360
361	DHSLPQDGSN AFISQKQNSK SVPSAKVSLK EYRAKHAEEL AAQKRQLENM EANVKSQYAY	420
421	AAQNLLSHHD SHSSVILKMP IEGSENPERP FLEKADKTAL KMRIPVAGGD KAASSKPEEI	480
481	KMRIKVHAAA DKHNSVEDSV TKSREHKEKH KTHPSNHHHH HNHHSHKHSH SQLPVGTGNK	540
541	RPGDPKHSSQ TSNLAHKTYS LSSSFSSSSS TRKRGPSEET GGAVFDHPAK IAKSTKSSSL	600
601	NFSFPSLPTM GQMPGHSSDT SGLSFSQPSC KTRVPHSKLD KGPTGANGHN TTQTIDYQDT	660
661	VNMLHSLLSA QGVQPTQPTA FEFVRPYSDY LNPRSGGISS RSGNTDKPRP PPLPSEPPPP	720
721	LPPLPK	780

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

[2]NCBI/Protein accession number NP_001231.2
HGNC identifier CycT1: CCNT1

Recombinant Proteins