Certificate of Analysis

REACTION BIOLOGY

ProQinase™ CDK8/CycC

cyclin-dependent kinase 8 / cyclin C

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK8

Synonyms: K35

Product No.: 0376-0390-1

Lot: 004

Description: Human CDK8, full length, amino acids M_1-Y_{463} (as in NCBI/Protein entry XP_005266272.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site and human CycC, full length, amino acids M_1-S_{283} (as in NCBI/Protein entry NP_005181.2), N-terminally fused to HIS_6-Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK8/CycC Lot004 was confirmed as CDK8/CycC by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK8}$: 83,051 Da
Theoretical $MW_{His\text{-}CycC}$: 37,999 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was not activated by special procedures

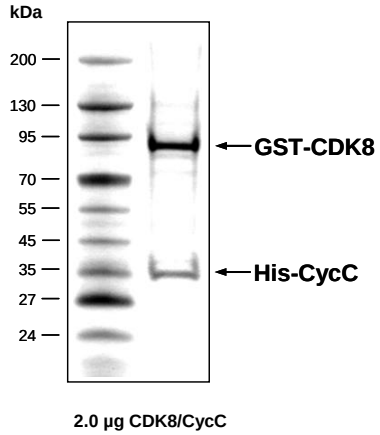
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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

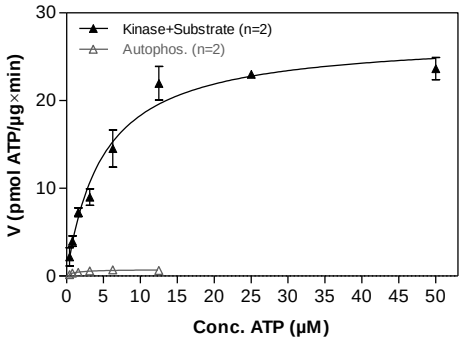
Biochemical Parameters:
Specific kinase activity (P_i transfer): 27 pmol/µg × min
ATP-K_M: 4.9 µM

CDK8/CycC Lot 004:
Coomassie stain



2.0 µg CDK8/CycC

CDK8/CycC 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-IRStide, 100 µg/ml
CDK8/CycC: 2 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

Recombinant Proteins

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ProQinase™ CDK8/CycC

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Recombinant Proteins

GST-CDK8 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 GIYARGIQAS MDYDFKVKLS SERERVEDLF EYEGCKVGRG TYGHVYKAKR	300
301	KDGKDDKDYA LKQIEGTGIS MSACREIALL RELKHPNVIS LQKVFLSHAD RKVWLLFDYA	360
361	EHDLWHIIKF HRASKANKKP VQLPRGMVKS LLYQILDGIH YLHANWVLHR DLKPANILVM	420
421	GEGPERGRVK IADMGFARLF NSPLKPLADL DPVVVTFWYR APELLLGARH YTKAIDIWAI	480
481	GCIFAELLTS EPIFHCRQED IKTSNPYHHD QLDRIFNVMG FPADKDWEDI KKMPEHSTLM	540
541	KDFRRNTYTN CSLIKYMEKH KVKPDSKAFH LLQKLLTMDP IKRITSEQAM QDPYFLEDPL	600
601	PTSDVFAGCQ IPYPKREFLT EEEPDDKGDK NQQQQQGNNH TNGTGHPGNQ DSSHTQGPPL	660
661	KKVRVVPPTT TSGGLIMTSD YQRSNPHAAY PNPGPSTSQP QSSMGYSATS QQPPQYSHQT	720
721	HRY	780

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDK8

CDK8 wt[1] Amino Acid Sequence

1	MDYDFKVKLS SERERVEDLF EYEGCKVGRG TYGHVYKAKR KDGKDDKDYA LKQIEGTGIS	60
61	MSACREIALL RELKHPNVIS LQKVFLSHAD RKVWLLFDYA EHDLWHIIKF HRASKANKKP	120
121	VQLPRGMVKS LLYQILDGIH YLHANWVLHR DLKPANILVM GEGPERGRVK IADMGFARLF	180
181	NSPLKPLADL DPVVVTFWYR APELLLGARH YTKAIDIWAI GCIFAELLTS EPIFHCRQED	240
241	IKTSNPYHHD QLDRIFNVMG FPADKDWEDI KKMPEHSTLM KDFRRNTYTN CSLIKYMEKH	300
301	KVKPDSKAFH LLQKLLTMDP IKRITSEQAM QDPYFLEDPL PTSDVFAGCQ IPYPKREFLT	360
361	EEEPDDKGDK NQQQQQGNNH TNGTGHPGNQ DSSHTQGPPL KKVRVVPPTT TSGGLIMTSD	420
421	YQRSNPHAAY PNPGPSTSQP QSSMGYSATS QQPPQYSHQT HRY	480

blue: CDK8 sequence expressed in recombinant protein

[1]NCBI/Protein accession number XP_005266272.1

HIS-CycC Recombinant Fusion Protein Amino Acid Sequence

1	MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGIYAR GIQASMAGNF WQSSHYLQWI	60
61	LDKQDLLKER QKDLKFLSEE EYWKLQIFFT NVIQALGEHL KLRQQVIATA TVYFKRFYAR	120
121	YSLKSIDPVL MAPTCVFLAS KVEEFGVVSN TRLIAAATSV LKTRFSYAFP KEFPYRMNHI	180
181	LECEFYLLEL MDCCLIVYHP YRPLLQYVQD MGQEDMLLPL AWRIVNDTYR TDLCLLYPPF	240
241	MIALACLHVA CVVQQKDARQ WFAELSVDME KILEIIRVIL KLYEQWKNFD ERKEMATILS	300
301	KMPKPKPPPN SEGEQGPNGS QNSSYSQS	360

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

CycC wt[1] Amino Acid Sequence

1	MAGNFWQSSH YLQWILDKQD LLKERQKDLK FLSEEEYWKL QIFFTNVIQA LGEHLKLRQQ	60
61	VIATATVYFK RFYARYSLKS IDPVLMAPTC VFLASKVEEF GVVSNTRLIA AATSVLKTRF	120
121	SYAFPKEFPY RMNHILECEF YLLELMDCCL IVYHPYRPLL QYVQDMGQED MLLPLAWRIV	180
181	NDTYRTDLCL LYPPFMIALA CLHVACVVQQ KDARQWFAEL SVDMEKILEI IRVILKLYEQ	240
241	WKNFDERKEM ATILSKMPKP KPPPNSEGEQ GPNGSQNSSY SQS	300

blue: CycC sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005181.2