

Certificate of Analysis



CDK3/CycC

cyclin-dependent kinase 3/Cyclin C

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK3

Synonyms: CDKN3

Product No.: 0212-0390-1

Lot: 004

Description: Human CDK3, full length, amino acids M₁-H₃₀₅ (as in NCBI/Protein entry NP_001249.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site and human CycC, full length, amino acids M₁-S₂₈₃ (as in NCBI/Protein entry NP_005181.2), N-terminally fused to HIS₆-Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK3/CycC Lot 004, was confirmed as CDK3/CycC by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-CDK3}: 68,193 Da

Theoretical MW_{HIS-CycC}: 37,999 Da

Expression: Baculovirus infected Sf9 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: 0.182 µg/µl

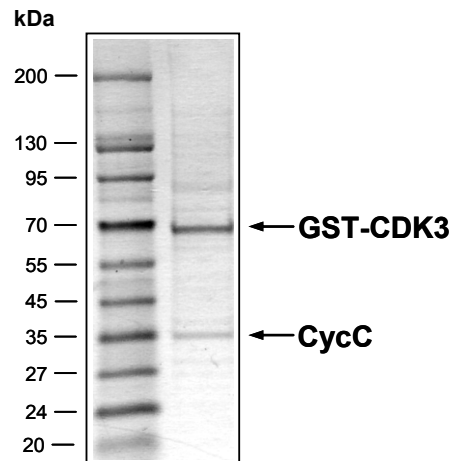
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

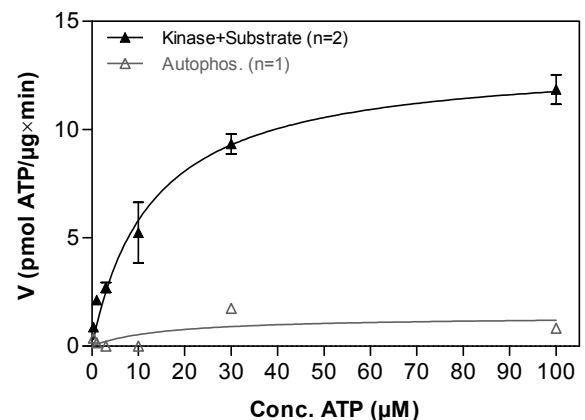
Specific kinase activity (P_i transfer): 13 pmol/µg×min

ATP-K_M: 13 µM

CDK3/CycC Lot 004:
Coomassie stain



CDK3/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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: RBER-CHKtide, 40 µg/ml
 - CDK3/CycC: 2.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: CDK3/CycC, Lot 004

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega



CDK3/CycC

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CDK3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRG SPGLD	GIYARGIQAS	MGARGRQCDG	YLQNSPLAMD	MFQKVEKIGE	GTYGVVYKAK	300
301	NRETGQVAL	KKIRLDLEME	GVPSTAI REI	SLLKELKHPN	IVRLLDVVHN	ERKLYLVFEF	360
361	LSQDLKMYD	STPGSELPLH	LIKSYLFQLL	QGVSFCHSHR	VIHRDLKPQN	LLINELGAIK	420
421	LADFG LARAF	GVPLR TYTHE	VVTLWYRAPE	ILLGSKFYTT	AVDIWSIGCI	FAEMVTRKAL	480
481	FPGDSEIDQL	FRIFRMLGTP	SEDTWPGVTQ	LPDYKGSFPK	WTRKGLEEIV	PNLEPEGRDL	540
541	LMQLLQYDPS	QRITAKTALA	HPYFSSPEPS	PAARQYVLQR	FRHGKGEFQH	TGARY	600

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** CDK3

CDK3 wt ¹ Amino Acid Sequence							
1	MDMFQKVEKI	GEGTYGVVYK	AKNRETGQLV	ALKKIRLDLE	MEGVPSTAIR	EISLLKELKH	60
61	PNIVRLLDVV	HNERKLYLVF	EFLSQDLKKY	MDSTPGSELP	LHLIKSYLFQ	LLQGVSFCHS	120
121	HRVIHRDLKP	QNLLINELGA	IKLADFG LAR	AFGVPLR TYT	HEVVTLWYRA	PEILLGSKFY	180
181	TTAVDIWSIG	CIFAEMVTRK	ALFPGDSEID	QLFRIFRMLG	TPSEDTWPGV	TQLPDYKGSF	240
241	PKWTRKLEE	IVPNLEPEGR	DLLMQLLQYD	PSQRITAKTA	LAHPYFSSPE	PSPAARQYVL	300
301	QRFRH						360

blue: CDK3 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_001249.1

CycC Recombinant Fusion Protein Amino Acid Sequence							
1	MSPIDPMG HH	HHHH GRRRAS	VAAGI LVPRG	SPGLDGIYAR	GIQASMAGNF	WQSSHYLQWI	60
61	LDKQDLLKER	QKDLKFLSEE	EYWK LQIFFT	NVIQALGEHL	KLRQQVIATA	TVYFKRFYAR	120
121	YLSKSIDPVL	MAPTCVFLAS	KVEEFGVVS N	TRLIAAATS V	LKTRFSYAFP	KEFPYRMNHI	180
181	LECEFY LLEL	MDCCLIVYHP	YRPLLQYVQD	MGQEDMLLPL	AWRIVNDTYR	TDLCLLYPPF	240
241	MIALACLHVA	CVVQQKDARQ	WFAELSVDME	KILEIIRVIL	KLYEQWKNFD	ERKEMATILS	300
301	KMPKPKPPP N	SEGEQGPNGS	QNSSYSQS				360

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

CycC wt ² Amino Acid Sequence							
1	MAGNFWQSSH	YLQWILDKQD	LLKERQKDLK	FLSEEEYWK L	QIFFT NVIQA	LGEHLKLRQQ	60
61	VIATATVYFK	RFYARYSLKS	IDPVLMAPTC	VFLASKVEEF	GVSNTRLIA	AATSVLKTRF	120
121	SYAFPKEFPY	RMNHILECEF	YLLELMDCCL	IVYHYP RPLL	QYVQDMGQED	MLLPLAWRIV	180
181	NDTYRTD LCL	LYPPFMIALA	CLHVACVVQQ	KDARQWFAEL	SVDMEKILEI	IRVILKLYEQ	240
241	WKNFDERKEM	ATILSKMPKP	KPPNSEGEQ	GPNGSQNSSY	SQS		300

blue: CycC sequence expressed in fusionprotein

²NCBI/Protein accession number NP_005181.2