Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ CDK1/CycE1

cyclin dependent kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK1

Synonyms: CDC2

Product No.: 0134-0055-1

Lot: 001

Description: Human CDK1, full length, amino acids M_1-M_{297} (as in NCBI/Protein entry NP_001777.1) and human CycE1, amino acids M_1-A_{395} (as in NCBI/Protein entry NP_476530.1), both N-terminally fused to GST-Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK1/CycE1 Lot 001, was confirmed as CDK1/CycE1 by specific Western Blotting using anti CDK1 and CycE1 antibodies

Theoretical MW_{CDK1}: 63,882 Da
Theoretical MW_{CycE1}: 72,041 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

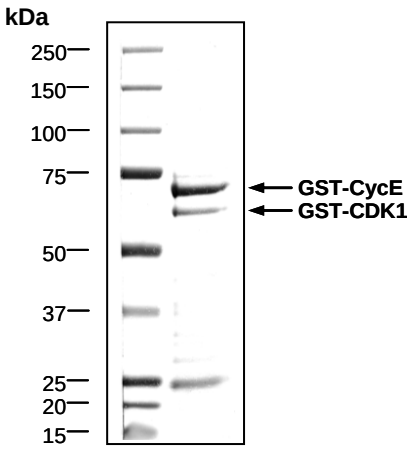
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

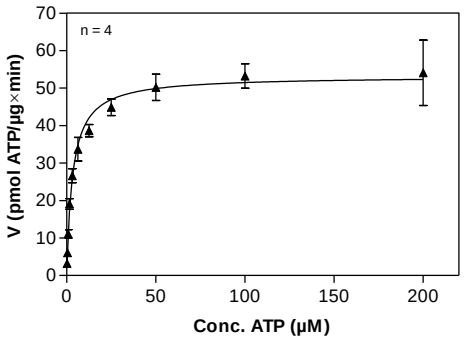
Biochemical Parameters:
Specific kinase activity (P_i transfer): 53 pmol/µg × min
ATP-K_M: 3.3 µM

CDK1/CycE1 Lot 001:
Coomassie stain



2.0 µg CDK1/CycE

CDK1/CycE1 Lot 001:
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: RB-CTF, 100 µg/ml
  CDK1/CycE1: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
CDK1/CycE1 Lot 001 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

ProQinase™ CDK1/CycE1

Product No.: 0134-0055-1

GST-CDK1 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 GICSIEEFTM EDYTKIEKIG EGTYGVVYKG RHKTTGQVVA MKKIRLESGE	300
301	EGVPSTAIRE ISLLKELRHP NIVSLQDVLM QDSRLYLIFE FLSMDLKKYL DSIPPGQYMD	360
361	SSLVKSYLYQ ILQGIVFCHS RRVLHRDLKP QNLLIDDKGT IKLADFGLAR AFGIPIRVYT	420
421	HEVVTLWYRS PEVLLGSARY STPVDIWSIG TIFAELATKK PLFHGDSEID QLFRIFRALG	480
481	TPNNEVWPEV ESLQDYKNTF PKWKPGSLAS HVKNLDENGL DLLSKMLIYD PAKRISGKMA	540
541	LNHPYFNDLD NQIKKM	600

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDK1 **boxed**: variation from RefSeq

CDK1 wt[1] Amino Acid Sequence

1	MEDYTKIEKI GEGTYGVVYK GRHKTTGQVV AMKKIRLESE EEGVPSTAIR EISLLKELRH	60
61	PNIVSLQDVL MQDSRLYLIF EFLSMDLKKY LDSIPPGQYM DSSLVKSYLY QILQGIVFCH	120
121	SRRVLHRDLK PQNLLIDDKG TIKLADFGLA RAFGIPIRVY THEVVTLWYR SPEVLLGSAR	180
181	YSTPVDIWSI GTIFAELATK KPLFHGDSEI DQLFRIFRAL GTPNNEVWPE VESLQDYKNT	240
241	FPKWKPGSLA SHVKNLDENG LDLLSKMLIY DPAKRISGKM ALNHPYFNDL DNQIKKM	300

blue: CDK1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_001777.1

GST-CycE1 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 LVPRGSPERD TMKEDGGAEF	240
241	SARSRKRKAN VTVFLQDPDE EMAKIDRTAR DQCGSQPWDN NAVCADPCSL IPTPDKEDDD	300
301	RVYPNSTCKP RIIAPSRGSP LPVLSWANRE EVWKIMLNKE KTYLRDQHFL EQHPLLQPKM	360
361	RAILLDWLME VCEVYKLHRE TFYLAQDFFD RYMATQENVV KTLLQLIGIS SLFIAAKLEE	420
421	IYPPKLHQFA YVTDGACSGD EILTMELMIM KALKWRLSPL TIVSWLNVYM QVAYLNDLHE	480
481	VLLPQYPQQI FIQIAELLDL CVLDVDCLEF PYGILAASAL YHFSSSELMQ KVSGYQWCDI	540
541	ENCVKWMVPF AMVIRETGSS KLKHFRGVAD EDAHNIQTHR DSLDLLDKAR AKKAMLSEQN	600
601	RASPLPSGLL TPPQSGKKQS SGPEMA	660

1-218: GST **Pink**: Thrombin cleavage site **blue**: CycE1

Kinase wt[2] Amino Acid Sequence

1	MKEDGGAEFS ARSRKRKANV TVFLQDPDEE MAKIDRTARD QCGSQPWDNN AVCADPCSLI	60
61	PTPDKEDDDR VYPNSTCKPR IIAPSRGSPL PVLSWANREE VWKIMLNKEK TYLRDQHFLE	120
121	QHPLLQPKMR AILLDWLMEV CEVYKLHRET FYLAQDFFDR YMATQENVVK TLLQLIGISS	180
181	LFIAAKLEEI YPPKLHQFAY VTDGACSGDE ILTMELMIMK ALKWRLSPLT IVSWLNVYMQ	240
241	VAYLNDLHEV LLPQYPQQIF IQIAELLDLC VLDVDCLEFP YGILAASALY HFSSSELMQK	300
301	VSGYQWCDIE NCVKWMVPFA MVIRETGSSK LKHFRGVADE DAHNIQTHRD SLDLLDKARA	360
361	KKAMLSEQNR ASPLPSGLLT PPQSGKKQSS GPEMA	420

blue: CycE1 sequence expressed in recombinant protein

[2]NCBI/Protein accession number NP_476530.1
HGNC symbol: CCNE1

Recombinant Proteins