Certificate of Analysis

REACTION BIOLOGY

ProQinase™ CDK1/CycE2

cyclin dependent kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK1

Synonyms: CDC2

Product No.: 0134-2123-1

Lot: 001

Description: Human CDK1, full length, amino acids M_1-M_{297} (as in NCBI/Protein entry NP_001777.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, and human CycE2, full length, amino acids M1-H404 (as in NCBI/Protein entry NP_477097.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site expressed in Sf9 insect cells

Product identity: CDK1/CycE2 Lot 001, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK1}$:	63882	Da
Theoretical $MW_{GST\text{-}CycE2}$:	74847	Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

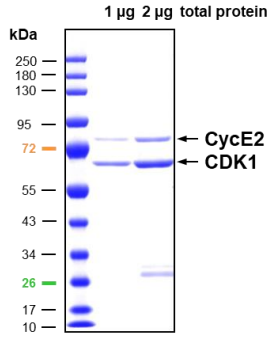
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

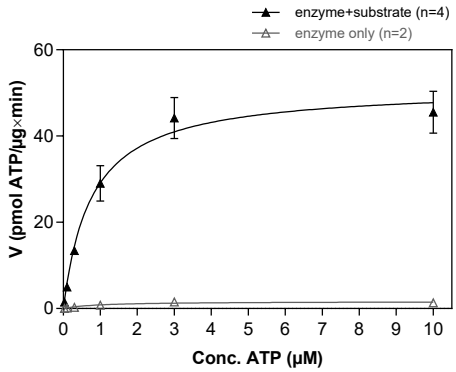
Protein concentration: 0.473 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 51 pmol/µg*min
ATP-K_M: 0.8 µM

CDK1/CycE2 Lot 001:
Coomassie stain



CDK1/CycE2 Lot 001:
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-GSK3 80 µg/ml
  Kinase: 0.5 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

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REACTION BIOLOGY

Recombinant Proteins

Sequence information

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

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

[1]NCBI/Protein accession number NP_001777.1

GST-CycE2 Recombinant Fusion Protein Amino Acid Sequence

```
  1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID   60
 61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV  120
121  DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK  180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG  240
241  PMSRRSSRLQ AKQQPQPSQT ESPQEAQIIQ AKKRKTTQDV KKRREEVTKK HQYEIRNCWP  300
301  PVLSGGISPC IIIETPHKEI GTSDFSRFTN YRFKNLFINP SPLPDLSWGC SKEVWLNMLK  360
361  KESRYVHDKH FEVLHSDLEP QMRSILLDWL LEVCEVYTLH RETFYLAQDF FDRFMLTQKD  420
421  INKNMLQLIG ITSLFIASKL EEIYAPKLQE FAYVTDGACS EEDILRMELI ILKALKWELC  480
481  PVTIISWLNL FLQVDALKDA PKVLLPQYSQ ETFIQIAQLL DLCILAIDSL EFQYRILTAA  540
541  ALCHFTSIEV VKKASGLEWD SISECVDWMV PFVNVVKSTS PVKLKTFKKI PMEDRHNIQT  600
601  HTNYLAMLEE VNYINTFRKG GQLSPVCNGG IMTPPKSTEK PPGKH                 660
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CycE2

CycE2 wt[2] Amino Acid Sequence

```
  1  MSRRSSRLQA KQQPQPSQTE SPQEAQIIQA KKRKTTQDVK KRREEVTKKH QYEIRNCWPP   60
 61  VLSGGISPCI IIETPHKEIG TSDFSRFTNY RFKNLFINPS PLPDLSWGCS KEVWLNMLKK  120
121  ESRYVHDKHF EVLHSDLEPQ MRSILLDWLL EVCEVYTLHR ETFYLAQDFF DRFMLTQKDI  180
181  NKNMLQLIGI TSLFIASKLE EIYAPKLQEF AYVTDGACSE EDILRMELII LKALKWELCP  240
241  VTIISWLNLF LQVDALKDAP KVLLPQYSQE TFIQIAQLLD LCILAIDSLE FQYRILTAAA  300
301  LCHFTSIEVV KKASGLEWDS ISECVDWMVP FVNVVKSTSP VKLKTFKKIP MEDRHNIQTH  360
361  TNYLAMLEEV NYINTFRKGG QLSPVCNGGI MTPPKSTEKP PGKH                   420
```

blue: CycE2 sequence expressed in recombinant protein

[2]NCBI/Protein accession number NP_477097.1
HGNC identifier CycE2: CCNE2

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