Certificate of Analysis

REACTION BIOLOGY

ProQinase™ CDK2/CycE2

cyclin dependent kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK2

Synonyms: p33

Product No.: 0050-2123-1

Lot: 001

Description: Human CDK2, full length, amino acids M_1-L_{298} (as in NCBI/Protein entry NP_001789.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, and human CycE2, amino acids M_1-H_{404} (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: CDK2/CycE2 Lot 001, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK2}$:	60222	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

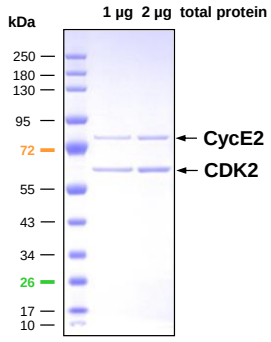
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

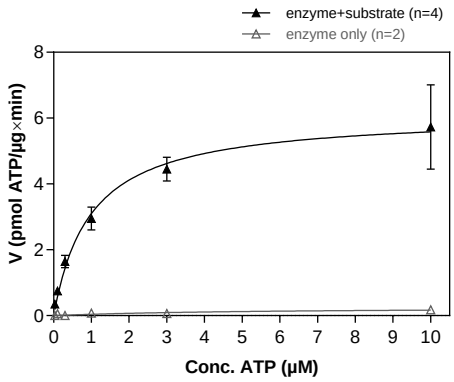
Protein concentration: 0.583 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 6.1 pmol/µg*min
ATP-K_M: 1 µM

CDK2/CycE2 Lot001:
Coomassie stain



CDK2/CycE2 Lot001:
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: Histone H1 10 µg/ml
  Kinase: 1 µg/ml

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

Recombinant Proteins

Sequence information

GST-CDK2 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 LVPRGSMENF QKVEKIGEGT 240
241  YGVVYKARNK LTGEVVALKK IRLDTETEGV PSTAIREISL LKELNHPNIV KLLDVIHTEN 300
301  KLYLVFEFLH QDLKKFMDAS ALTGIPLPLI KSYLFQLLQG LAFCHSHRVL HRDLKPQNLL 360
361  INTEGAIKLA DFGLARAFGV PVRTYTHEVV TLWYRAPEIL LGCKYYSTAV DIWSLGCIFA 420
421  EMVTRRALFP GDSEIDQLFR IFRTLGTPDE VVWPGVTSMP DYKPSFPKWA RQDFSKVVPP 480
481  LDEDGRSLLS QMLHYDPNKR ISAKAALAHP FFQDVTKPVP HLRL                   540
```

1-218: GST **Pink**: Thrombin cleavage site **blue**: CDK2

CDK2 wt[1] Amino Acid Sequence

```
  1  MENFQKVEKI GEGTYGVVYK ARNKLTGEVV ALKKIRLDTE TEGVPSTAIR EISLLKELNH  60
 61  PNIVKLLDVI HTENKLYLVF EFLHQDLKKF MDASALTGIP LPLIKSYLFQ LLQGLAFCHS 120
121  HRVLHRDLKP QNLLINTEGA IKLADFGLAR AFGVPVRTYT HEVVTLWYRA PEILLGCKYY 180
181  STAVDIWSLG CIFAEMVTRR ALFPGDSEID QLFRIFRTLG TPDEVVWPGV TSMPDYKPSF 240
241  PKWARQDFSK VVPPLDEDGR SLLSQMLHYD PNKRISAKAA LAHPFFQDVT KPVPHLRL   300
```

blue: CDK2 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001789.2

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

Recombinant Proteins