Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ CDK1 D146N/CycB1

cyclin Dependent Kinase 1

Recombinant Kinase Substrate (Human Protein Kinase, point mutant, inactive)

HGNC Symbol: CDK1

Synonyms: CDC2, CDC28A

Product No.: 1830-0135-1

Lot: 003

Description: Human CDK1, amino acids M_1-M_{297} (as in NCBI/Protein entry NP_001777.1) with a point mutation D146N (resulting in loss of kinase activity) and human CycB1, amino acids M1-V433 (as in NCBI/Protein entry NP_114172.1), both N-terminal GST-HIS_6 fusion proteins with a 3C (CDK1) and Thrombin (CycB1) cleavage site, coexpressed in Sf9 insect cells

Theoretical $MW_{GST\text{-}CDK1}$: 62,997 Da
Theoretical $MW_{GST\text{-}CycB1}$: 78,919 Da

Expression host: Sf9 insect cells

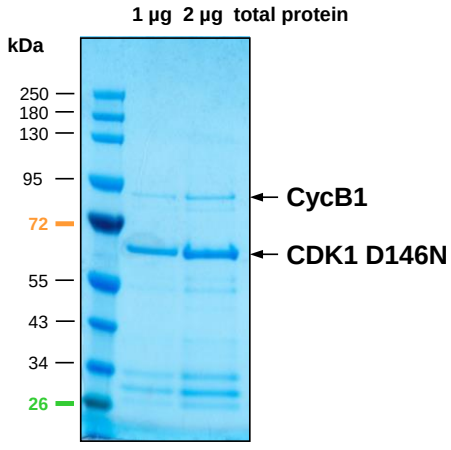
Purification: GST Affinity Chromatography

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

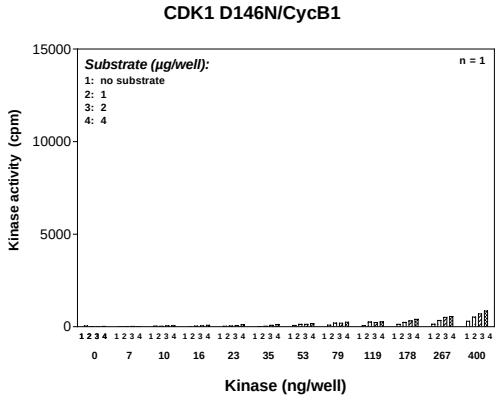
Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

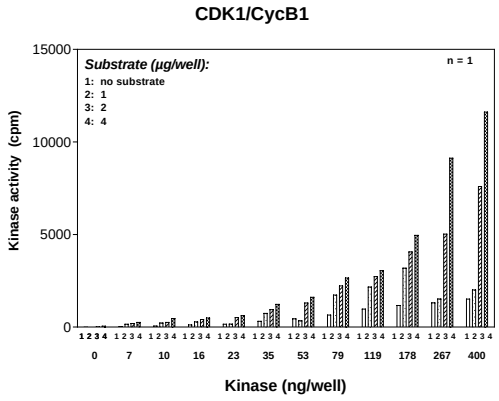
Protein concentration: 3.093 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

CDK1 D146N/CycB1 Lot 003: Coomassie stain



Kinase activity CDK1 D146N/CycB1 vs. CDK1/CycB1





- 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-CHKtide (variable amounts)
  Kinase: variable amounts

Assay: ^{33}PanQinase® Assay

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CDK1 D146N/CycB1

Product No.: 1830-0135-1

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 KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241  PLAMGARGRM EDYTKIEKIG EGTYGVVYKG RHKTTGQVVA MKKIRLESEE EGVPSTAIRE 300
301  ISLLKELRHP NIVSLQDVLM QDSRLYLIFE FLSMDLKKYL DSIPPGQYMD SSLVKSYLYQ 360
361  ILQGIVFCHS RRVLHRDLKP QNLLIDDKGT IKLANFGLAR AFGIPIRVYT HEVVTLWYRS 420
421  PEVLLGSARY STPVDIWSIG TIFAELATKK PLFHGDSEID QLFRIFRALG TPNNEVWPEV 480
481  ESLQDYKNTF PKWKPGSLAS HVKNLDENGL DLLSKMLIYD PAKRISGKMA LNHPYFNDLD 540
541  NQIKKM                                                          600
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CDK1 **boxed**: D146N

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-CycB1 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 GICSIEEFRP PWELAMALRV TRNSKINAEN KAKINMAGAK RVPTAPAATS 300
301  KPGLRPRTAL GDIGNKVSEQ LQAKMPMKKE AKPSATGKVI DKKLPKPLEK VPMLVPVPVS 360
361  EPVPEPEPEP EPEPVKEEKL SPEPILVDTA SPSPMETSGC APAEEDLCQA FSDVILAVND 420
421  VDAEDGADPN LCSEYVKDIY AYLRQLEEEQ AVRPKYLLGR EVTGNMRAIL IDWLVQVQMK 480
481  FRLLQETMYM TVSIIDRFMQ NNCVPKKMLQ LVGVTAMFIA SKYEEMYPPE IGDFAFVTDN 540
541  TYTKHQIRQM EMKILRALNF GLGRPLPLHF LRRASKIGEV DVEQHTLAKY LMELTMSDYD 600
601  MVHFPPSQIA AGAFCLALKI LDNGEWTPTL QHYLSYTEES LLPVMQHLAK NVVMVNQGLT 660
661  KHMTVKNKYA TSKHAKISTL PQLNSALVQD LAKAVAKV                        720
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycB1 **boxed**: variation from RefSeq

CycB1 wt[2] Amino Acid Sequence

```
1    MALRVTRNSK INAENKAKIN MAGAKRVPTA PAATSKPGLR PRTALGDIGN KVSEQLQAKM 60
61   PMKKEAKPSA TGKVIDKKLP KPLEKVPMLV PVPVSEPVPE PEPEPEPEPV KEEKLSPEPI 120
121  LVDTASPSPM ETSGCAPAEE DLCQAFSDVI LAVNDVDAED GADPNLCSEY VKDIYAYLRQ 180
181  LEEEQAVRPK YLLGREVTGN MRAILIDWLV QVQMKFRLLQ ETMYMTVSII DRFMQNNCVP 240
241  KKMLQLVGVT AMFIASKYEE MYPPEIGDFA FVTDNTYTKH QIRQMEMKIL RALNFGLGRP 300
301  LPLHFLRRAS KIGEVDVEQH TLAKYLMELT MLDYDMVHFP PSQIAAGAFC LALKILDNGE 360
361  WTPTLQHYLS YTEESLLPVM QHLAKNVVMV NQGLTKHMTV KNKYATSKHA KISTLPQLNS 420
421  ALVQDLAKAV AKV                                                   480
```

blue: CycB1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[2]NCBI/Protein accession number NP_114172.1

Recombinant Proteins