Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

CDK6/CycD1

cyclin dependent kinase 6

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK6

Synonyms: PLSTIRE; cell division protein kinase 6

Product No.: 0051-0143-2

Lot: 006

Description: Human CDK6, full length, amino acids M_1-A_{326} (NCBI/Protein entry NP_001250.1), N-terminally fused to GST-Thrombin cleavage site and human CycD1, full length, amino acids Q_4-I_{295} (NCBI/Protein entry NP_444284.1), N-terminally fused to GST-Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CDK6/CycD1 Lot 006, was confirmed as CDK6/CycD1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK6}$: 63,279 Da
Theoretical $MW_{GST\text{-}CycD1}$: 59,995 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro by CAK1

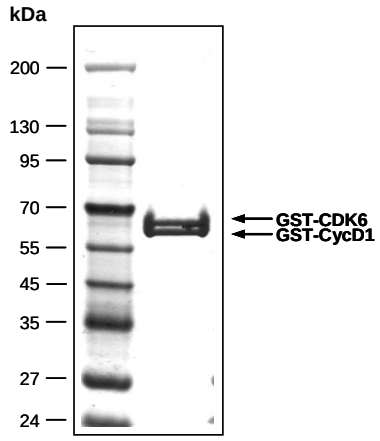
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

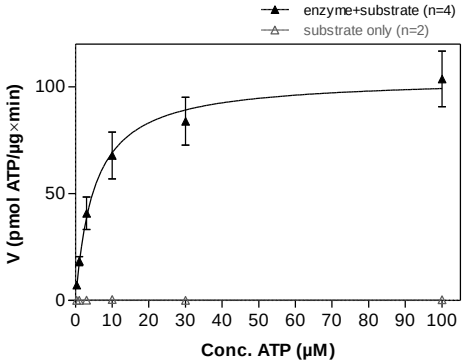
Biochemical Parameters:
Specific kinase activity (P_i transfer): 104 pmol/µg × min
ATP-K_M: 8.1 µM

CDK6/CycD1 Lot 006:
Coomassie stain



2.0 µg GST-CDK6/GST-CycD1

CDK6/CycD1 Lot 006:
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
  Kinase: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
CDK6/CycD1 Lot 006 was also successfully tested by ProQinase 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
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CDK6/CycD1

Product No.: 0051-0143-2

GST-CDK6 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 LVPRGSMEKD GLCRADQQYE 240
241  CVAEIGEGAY GKVFKARDLK NGGRFVALKR VRVQTGEEGM PLSTIREVAV LRHLETFEHP 300
301  NVVRLFDVCT VSRTDRETKL TLVFEHVDQD LTTYLDKVPE PGVPTETIKD MMFQLLRGLD 360
361  FLHSHRVVHR DLKPQNILVT SSGQIKLADF GLARIYSFQM ALTSVVVTLW YRAPEVLLQS 420
421  SYATPVDLWS VGCIFAEMFR RKPLFRGSSY VDQLGKILDV IGLPGEEDWP RDVALPRQAF 480
481  HSKSAQPIEK FVTDIDELGK DLLLKCLTFN PAKRISAYSA LSHPYFQDLE RCKENLDSHL 540
541  PPSQNTSELN TA                                                   600
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDK6 **boxed**: variation from RefSeq

CDK6 wt[1] Amino Acid Sequence

```
1    MEKDGLCRAD QQYECVAEIG EGAYGKVFKA RDLKNGGRFV ALKRVRVQTG EEGMPLSTIR 60
61   EVAVLRHLET FEHPNVVRLF DVCTVSRTDR ETKLTLVFEH VDQDLTTYLD KVPEPGVPTE 120
121  TIKDMMFQLL RGLDFLHSHR VVHRDLKPQN ILVTSSGQIK LADFGLARIY SFQMALTSVV 180
181  VTLWYRAPEV LLQSSYATPV DLWSVGCIFA EMFRRKPLFR GSSDVDQLGK ILDVIGLPGE 240
241  EDWPRDVALP RQAFHSKSAQ PIEKFVTDID ELGKDLLLKC LTFNPAKRIS AYSALSHPYF 300
301  QDLERCKENL DSHLPPSQNT SELNTA                                     360
```

blue: CDK6 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_001250.1

GST-CycD1 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 LVPRGSAWIQ LLCCEVETIR 240
241  RAYPDANLLN DRVLRAMLKA EETCAPSVSY FKCVQKEVLP SMRKIVATWM LEVCEEQKCE 300
301  EEVFPLAMNY LDRFLSLEPV KKSRLQLLGA TCMFVASKMK ETIPLTAEKL CIYTDNSIRP 360
361  EELLQMELLL VNKLKWNLAA MTPHDFIEHF LSKMPEAEEN KQIIRKHAQT FVALCATDVK 420
421  FISNPPSMVA AGSVVAAVQG LNLRSPNNFL SYYRLTRFLS RVIKCDPDCL RACQEQIEAL 480
481  LESSLRQAQQ NMDPKAAEEE EEEEEEVDLA CTPTDVRDVD I                   540
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycD1

CycD1 wt[2] Amino Acid Sequence

```
1    MEHQLLCCEV ETIRRAYPDA NLLNDRVLRA MLKAEETCAP SVSYFKCVQK EVLPSMRKIV 60
61   ATWMLEVCEE QKCEEEVFPL AMNYLDRFLS LEPVKKSRLQ LLGATCMFVA SKMKETIPLT 120
121  AEKLCIYTDN SIRPEELLQM ELLLVNKLKW NLAAMTPHDF IEHFLSKMPE AEENKQIIRK 180
181  HAQTFVALCA TDVKFISNPP SMVAAGSVVA AVQGLNLRSP NNFLSYYRLT RFLSRVIKCD 240
241  PDCLRACQEQ IEALLESSLR QAQQNMDPKA AEEEEEEEEE VDLACTPTDV RDVDI     300
```

blue: CycD1 sequence expressed in recombinant protein

[2]NCBI/Protein accession number NP_444284.1
HGNC identifier CycD1: CCND1

Recombinant Proteins