Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

CDK19/CycC

cyclin dependent kinase 19 / cyclin C

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK19

Synonyms: CDC2L6, CDK11

Product No.: 1384-0390-1

Lot: 016

Description: Human CDK19, amino acids M_1-Y_{502} (as in NCBI/Protein entry NP_055891.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site and human CycC, amino acids M_1-S_{283} (as in NCBI/Protein entry NP_005181.2), N-terminally fused to HIS_6-Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK19/CycC Lot 016, was confirmed as CDK19/CycC by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}CDK19}$: 84,892 Da
Theoretical MW_{CycC} : 37,999 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was not activated by special procedures

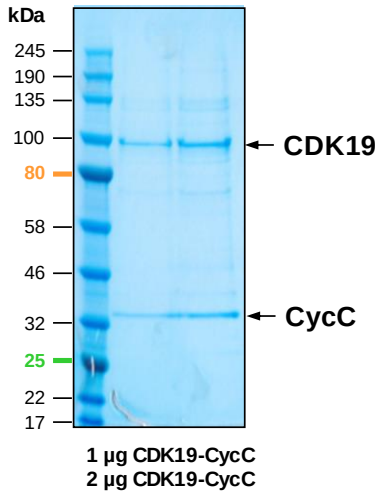
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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!

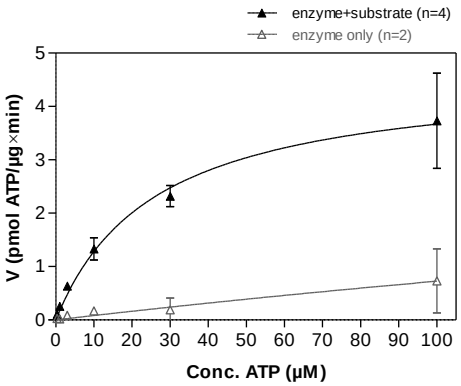
Protein concentration: 0.186 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 4.6 pmol/µg × min
ATP-K_M: 26 µM

CDK19/CycC Lot 016:
Coomassie stain



CDK19/CycC Lot 016:
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: RBER-IRStide, 80 µg/ml
Kinase: 2 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

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GST-CDK19 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 PMDYDFKAKL AAERERVEDL FEYEGCKVGR GTYGHVYKAR RKDGKDEKEY ALKQIEGTGI 300
 301 SMSACREIAL LRELKHPNVI ALQKVFLSHS DRKVWLLFDY AEHDLWHIIK FHRASKANKK 360
 361 PMQLPRSMVK SLLYQILDGI HYLHANWVLH RDLKPANILV MGEGPERGRV KIADMGFARL 420
 421 FNSPLKPLAD LDPVVVTFWY RAPELLLGAR HYTKAIDIWA IGCIFAELLT SEPIFHCRQE 480
 481 DIKTSNPFHH DQLDRIFSVM GFPADKDWED IRKMPEYPTL QKDFRRTTYA NSSLIKYMEK 540
 541 HKVKPDSKVF LLLQKLLTMD PTKRITSEQA LQDPYFQEDP LPTLDVFAGC QIPYPKREFL 600
 601 NEDDPEEKGD KNQQQQQNQH QQPTAPPQQA AAPPQAPPPQ QNSTQTNGTA GGAGAGVGGT 660
 661 GAGLQHSQDS SLNQVPPNKK PRLGPSGANS GGPVMPSDYQ HSSSRLNYQS SVQGSSQSQS 720
 721 TLGYSSSSQQ SSQYHPSHQA HRY                                         780
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CDK19

CDK19 wt[1] Amino Acid Sequence

```
1   MDYDFKAKLA AERERVEDLF EYEGCKVGRG TYGHVYKARR KDGKDEKEYA LKQIEGTGIS  60
61  MSACREIALL RELKHPNVIA LQKVFLSHSD RKVWLLFDYA EHDLWHIIKF HRASKANKKP 120
121 MQLPRSMVKS LLYQILDGIH YLHANWVLHR DLKPANILVM GEGPERGRVK IADMGFARLF 180
181 NSPLKPLADL DPVVVTFWYR APELLLGARH YTKAIDIWAI GCIFAELLTS EPIFHCRQED 240
241 IKTSNPFHHD QLDRIFSVMG FPADKDWEDI RKMPEYPTLQ KDFRRTTYAN SSLIKYMEKH 300
301 KVKPDSKVFL LLQKLLTMDP TKRITSEQAL QDPYFQEDPL PTLDVFAGCQ IPYPKREFLN 360
361 EDDPEEKGDK NQQQQQNQHQ QPTAPPQQAA APPQAPPPQQ NSTQTNGTAG GAGAGVGGTG 420
421 AGLQHSQDSS LNQVPPNKKP RLGPSGANSG GPVMPSDYQH SSSRLNYQSS VQGSSQSQST 480
481 LGYSSSSQQS SQYHPSHQAH RY                                          540
```

blue: CDK19 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_055891.1

HIS-CycC Recombinant Fusion Protein Amino Acid Sequence

```
   1 MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGIYAR GIQASMAGNF WQSSHYLQWI  60
  61 LDKQDLLKER QKDLKFLSEE EYWKLQIFFT NVIQALGEHL KLRQQVIATA TVYFKRFYAR 120
 121 YSLKSIDPVL MAPTCVFLAS KVEEFGVVSN TRLIAAATSV LKTRFSYAFP KEFPYRMNHI 180
 181 LECEFYLLEL MDCCLIVYHP YRPLLQYVQD MGQEDMLLPL AWRIVNDTYR TDLCLLYPPF 240
 241 MIALACLHVA CVVQQKDARQ WFAELSVDME KILEIIRVIL KLYEQWKNFD ERKEMATILS 300
 301 KMPKPKPPPN SEGEQGPNGS QNSSYSQS                                    360
```

Red: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycC

CycC wt[2] Amino Acid Sequence

```
1   MAGNFWQSSH YLQWILDKQD LLKERQKDLK FLSEEEYWKL QIFFTNVIQA LGEHLKLRQQ  60
61  VIATATVYFK RFYARYSLKS IDPVLMAPTC VFLASKVEEF GVVSNTRLIA AATSVLKTRF 120
121 SYAFPKEFPY RMNHILECEF YLLELMDCCL IVYHPYRPLL QYVQDMGQED MLLPLAWRIV 180
181 NDTYRTDLCL LYPPFMIALA CLHVACVVQQ KDARQWFAEL SVDMEKILEI IRVILKLYEQ 240
241 WKNFDERKEM ATILSKMPKP KPPPNSEGEQ GPNGSQNSSY SQS                   300
```

blue: CycC sequence expressed in recombinant protein

[2]NCBI/Protein accession number NP_005181.2
HGNC symbol CycC: CCNC

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