Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

CDK18/CycY

cyclin dependent kinase 18

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK18

Synonyms: PCTAIRE, PCTAIRE3, PCTK3

Product No.: 1546-1619-1

Lot: 008

Description: Human CDK18, amino acids M_1-F_{474} (as in NCBI/Protein entry NP_997667.1) and human CyclinY, amino acids M_1-S_{341} (as in NCBI/Protein entry NP_659449.3), both N-terminal GST-HIS_6 fusion proteins with a 3C cleavage site, coexpressed in Sf9 insect cells.

Product identity: CDK18/CycY Lot 008, was confirmed as CDK18/CycY by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{CDK18} : 82,514 Da
Theoretical MW_{CycY} : 67,841 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

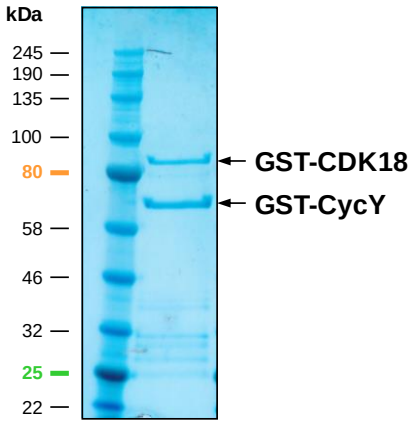
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

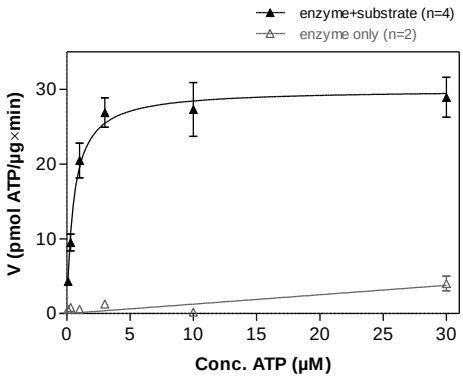
Biochemical Parameters:
Specific kinase activity (P_i transfer): 30 pmol/µg × min
ATP-K_M: 0.53 µM

CDK18/CycY Lot 008:
Coomassie stain



1 µg GST-CDK18/CycY

CDK18/CycY Lot 008:
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-NTRK3tide 4 µg/ml
  Kinase: 0.5 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
CDK18/CycY Lot 008 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

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CDK18/CycY

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GST-CDK18 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  PMIMNKMKNF KRRFSLSVPR TETIEESLAE FTEQFNQLHN RRNENLQLGP LGRDPPQECS  300
 301  TFSPTDSGEE PGQLSPGVQF QRRQNQRRFS MEDVSKRLSL PMDIRLPQEF LQKLQMESPD  360
 361  LPKPLSRMSR RASLSDIGFG KLETYVKLDK LGEGTYATVF KGRSKLTENL VALKEIRLEH  420
 421  EEGAPCTAIR EVSLLKNLKH ANIVTLHDLI HTDRSLTLVF EYLDSDLKQY LDHCGNLMSM  480
 481  HNVKIFMFQL LRGLAYCHHR KILHRDLKPQ NLLINERGEL KLADFGLARA KSVPTKTYSN  540
 541  EVVTLWYRPP DVLLGSTEYS TPIDMWGVGC IHYEMATGRP LFPGSTVKEE LHLIFRLLGT  600
 601  PTEETWPGVT AFSEFRTYSF PCYLPQPLIN HAPRLDTDGI HLLSSLLLYE SKSRMSAEAA  660
 661  LSHSYFRSLG ERVHQLEDTA SIFSLKEIQL QKDPGYRGLA FQQPGRGKNR RQSIF       720
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CDK18

CDK18 wt[1] Amino Acid Sequence

```
1    MIMNKMKNFK RRFSLSVPRT ETIEESLAEF TEQFNQLHNR RNENLQLGPL GRDPPQECST   60
61   FSPTDSGEEP GQLSPGVQFQ RRQNQRRFSM EDVSKRLSLP MDIRLPQEFL QKLQMESPDL  120
121  PKPLSRMSRR ASLSDIGFGK LETYVKLDKL GEGTYATVFK GRSKLTENLV ALKEIRLEHE  180
181  EGAPCTAIRE VSLLKNLKHA NIVTLHDLIH TDRSLTLVFE YLDSDLKQYL DHCGNLMSMH  240
241  NVKIFMFQLL RGLAYCHHRK ILHRDLKPQN LLINERGELK LADFGLARAK SVPTKTYSNE  300
301  VVTLWYRPPD VLLGSTEYST PIDMWGVGCI HYEMATGRPL FPGSTVKEEL HLIFRLLGTP  360
361  TEETWPGVTA FSEFRTYSFP CYLPQPLINH APRLDTDGIH LLSSLLLYES KSRMSAEAAL  420
421  SHSYFRSLGE RVHQLEDTAS IFSLKEIQLQ KDPGYRGLAF QQPGRGKNRR QSIF       480
```

blue: CDK18 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_997667.1

GST-CycY 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  PLAMVMGNTT SCCVSSSPKL RRNAHSRLES YRPDTDLSRE DTGCNLQHIS DRENIDDLNM  300
 301  EFNPSDHPRA STIFLSKSQT DVREKRKSLF INHHPPGQIA RKYSSCSTIF LDDSTVSQPN  360
 361  LKYTIKCVAL AIYYHIKNRD PDGRMLLDIF DENLHPLSKS EVPPDYDKHN PEQKQIYRFV  420
 421  RTLFSAAQLT AECAIVTLVY LERLLTYAEI DICPANWKRI VLGAILLASK VWDDQAVWNV  480
 481  DYCQILKDIT VEDMNELERQ FLELLQFNIN VPSSVYAKYY FDLRSLAEAN NLSFPLEPLS  540
 541  RERAHKLEAI SRLCEDKYKD LRRSARKRSA SADNLTLPRW SPAIIS                 600
```

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

CycY wt[2] Amino Acid Sequence

```
1    MGNTTSCCVS SSPKLRRNAH SRLESYRPDT DLSREDTGCN LQHISDRENI DDLNMEFNPS   60
61   DHPRASTIFL SKSQTDVREK RKSLFINHHP PGQIARKYSS CSTIFLDDST VSQPNLKYTI  120
121  KCVALAIYYH IKNRDPDGRM LLDIFDENLH PLSKSEVPPD YDKHNPEQKQ IYRFVRTLFS  180
181  AAQLTAECAI VTLVYLERLL TYAEIDICPA NWKRIVLGAI LLASKVWDDQ AVWNVDYCQI  240
241  LKDITVEDMN ELERQFLELL QFNINVPSSV YAKYYFDLRS LAEANNLSFP LEPLSRERAH  300
301  KLEAISRLCE DKYKDLRRSA RKRSASADNL TLPRWSPAII S                      360
```

blue: CycY sequence expressed in recombinant protein

[2]NCBI/Protein accession number NP_659449.3
HGNC identifier CycY: CCNY

Recombinant Proteins