Certificate of Analysis

REACTION BIOLOGY

ProQinase™ CDK15/CycB1

cyclin dependent kinase 15

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK15

Synonyms: PFTAIRE2; ALS2CR7; PFTK2

Product No.: 1889-0135-1

Lot: 002

Description: Human CDK15, internal fragment, amino acids Y_{103}-F_{387} (as in NCBI/Protein entry NP_001248364.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site and human cyclin B1, full length, amino acids M_1-V_{433} (as in NCBI/Protein entry NP_114172.4), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, co-expressed in Sf9 insect cells

Product identity: CDK15/CycB1 Lot 002, was confirmed as CDK15/CycB1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{CDK15}:	60,389	Da
Theoretical MW_{CycB1}:	78,919	Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

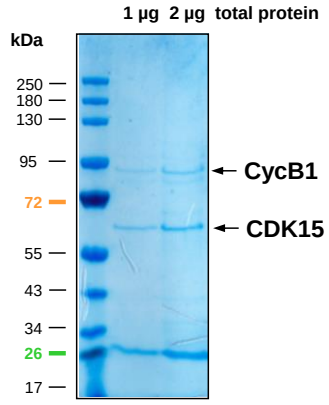
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

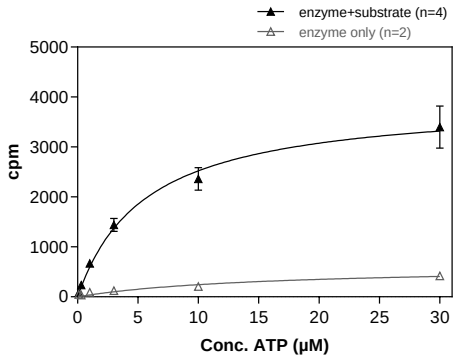
Protein concentration: 0.156 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 1.9 pmol/µg x min
ATP-K_M: 2.8 µM

CDK15/CycB1 Lot 002:
Coomassie stain



CDK15/CycB1 Lot 002:
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: RBER-NTRK3tide 80 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Recombinant Proteins

Sequence information

GST-CDK15 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  PYLNLEKLGE GSYATVYKGI SRINGQLVAL KVISMNAEEG VPFTAIREAS LLKGLKHANI 300
301  VLLHDIIHTK ETLTFVFEYM HTDLAQYMSQ HPGGLHPHNV RLFMFQLLRG LAYIHHQHVL 360
361  HRDLKPQNLL ISHLGELKLA DFGLARAKSI PSQTYSSEVV TLWYRPPDAL LGATEYSSEL 420
421  DIWGAGCIFI EMFQGQPLFP GVSNILEQLE KIWEVLGVPT EDTWPGVSKL PNYNPEWFPL 480
481  PTPRSLHVVW NRLGRVPEAE DLASQMLKGF PRDRVSAQEA LVHDYF              540
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: CDK15 fragment

CDK15 wt[1] Amino Acid Sequence

```
  1  MGQELCAKTV QPGCSCYHCS EGGEAHSCRR SQPETTEAAF KLTDLKEASC SMTSFHPRGL  60
 61  QAARAQKFKS KRPRSNSDCF QEEDLRQGFQ WRKSLPFGAA SSYLNLEKLG EGSYATVYKG 120
121  ISRINGQLVA LKVISMNAEE GVPFTAIREA SLLKGLKHAN IVLLHDIIHT KETLTFVFEY 180
181  MHTDLAQYMS QHPGGLHPHN VRLFMFQLLR GLAYIHHQHV LHRDLKPQNL LISHLGELKL 240
241  ADFGLARAKS IPSQTYSSEV VTLWYRPPDA LLGATEYSSE LDIWGAGCIF IEMFQGQPLF 300
301  PGVSNILEQL EKIWEVLGVP TEDTWPGVSK LPNYNPEWFP LPTPRSLHVV WNRLGRVPEA 360
361  EDLASQMLKG FPRDRVSAQE ALVHDYFSAL PSQLYQLPDV SGVRLKPEMC DLLASYQKGH 420
421  HPAQFSKCW                                                        480
```

blue: CDK15 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001248364.1

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

GST-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
HGNC identifier CycB1: CCNB1

Recombinant Proteins