Certificate of Analysis

Recombinant Proteins

ProQinase™ CDK17/p35NCK

Cyclin-dependent kinase 17

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK17

Synonyms: PCTAIRE2, PCTK2

Product No.: 1526-0355-1

Lot: 003

Description: Co-expression of human CDK17, amino acids M$_1$-F$_{523}$ (as in NCBI/Protein entry NP_002586.2), N-terminal GST-HIS$_6$ fusion protein with a 3C cleavage site and human p35NCK, amino acids M$_1$-R$_{307}$ (as in NCBI/Protein entry NP_003876.1), N-terminally fused to GST-Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CDK17/p35NCK Lot 003, was confirmed as CDK17/p35NCK by mass spectroscopy LC-ESI-MS/MS

Theoretical MW$_{GST\text{-}CDK17}$: 87,672 Da
Theoretical MW$_{GST\text{-}p35NCK}$: 63,956 Da

Expression: Baculovirus infected Sf9 cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was activated by coexpression with its physiological cofactor p35NCK

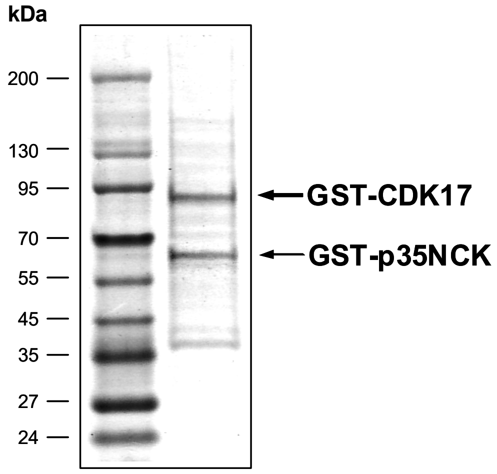
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20% glycerol

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.286 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

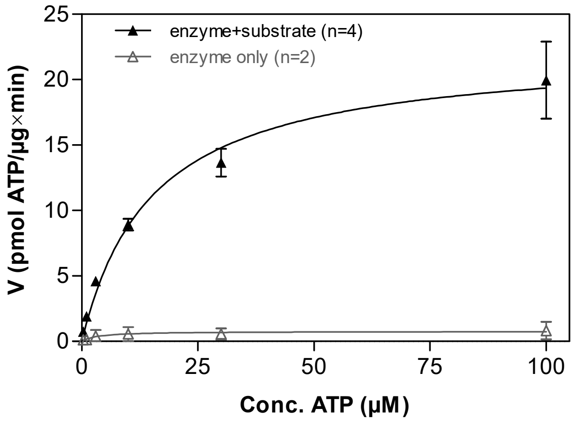
Biochemical Parameters:
Specific kinase activity (P_i transfer): 22 pmol/µg×min
ATP-K_M: 15.0 µM

CDK17/p35NCK Lot 003:
Coomassie stain



2.0 µg CDK17/p35NCK

CDK17/p35NCK Lot 003:
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: GSK3-derived peptide
  (R11-SGRARTSSFAEPGGK), 100 µg / ml
  CDK17/p35NCK: 4.0 µg / ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology: CDK17/p35NCK Lot 003 was also successfully tested by Reaction Biology for the use with the ADP-Glo^{TM} Kinase assay from ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase™ CDK17/p35NCK

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CDK17 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	PMG**HHHHHH**G	RDS**LEVLFQG**	240
241	**PMKKFKRRLS**	**LTLRGSQTID**	**ESLSELAEQM**	**TIEENSSKDN**	**EPIVKNGRPP**	**TSHSMHSFLH**	300
301	**QYTGSFKKPP**	**LRRPHSVIGG**	**SLGSFMAMPR**	**NGSRLDIVHE**	**NLKMGSDGES**	**DQASGTSSDE**	360
361	**VQSPTGVCLR**	**NRIHRRISME**	**DLNKRLSLPA**	**DIRIPDGYLE**	**KLQINSPPFD**	**QPMSRRSRRA**	420
421	**SLSEIGFGKM**	**ETYIKLEKLG**	**EGTYATVYKG**	**RSKLTENLVA**	**LKEIRLEHEE**	**GAPCTAIREV**	480
481	**SLLKDLKHAN**	**IVTLHDIVHT**	**DKSLTLVFEY**	**LDKDLKQYMD**	**DCGNIMSMHN**	**VKLFLYQILR**	540
541	**GLAYCHRRKV**	**LHRDLKPQNL**	**LINEKGELKL**	**ADFGLARAKS**	**VPTKTYSNEV**	**VTLWYRPPDV**	600
601	**LLGSSEYSTQ**	**IDMWGVGCIF**	**FEMASGRPLF**	**PGSTVEDELH**	**LIFRLLGTPS**	**QETWPGISSN**	660
661	**EEFKNYNFPK**	**YKPQPLINHA**	**PRLDSEGIEL**	**ITKFLQYESK**	**KRVSAEEAMK**	**HVYFRSLGPR**	720
721	**IHALPESVSI**	**FSLKEIQLQK**	**DPGFRNSSYP**	**ETGHGKNRRQ**	**SMLF**		780

1-218: GST **Red: HIS6-tag** **Green:** 3C cleavage site **blue**:CDK17

CDK17 wt[1] Amino Acid Sequence

1	**MKKFKRRLSL**	**TLRGSQTIDE**	**SLSELAEQMT**	**IEENSSKDNE**	**PIVKNGRPPT**	**SHSMHSFLHQ**	60
61	**YTGSFKKPPL**	**RRPHSVIGGS**	**LGSFMAMPRN**	**GSRLDIVHEN**	**LKMGSDGESD**	**QASGTSSDEV**	120
121	**QSPTGVCLRN**	**RIHRRISMED**	**LNKRLSLPAD**	**IRIPDGYLEK**	**LQINSPPFDQ**	**PMSRRSRRAS**	180
181	**LSEIGFGKME**	**TYIKLEKLGE**	**GTYATVYKGR**	**SKLTENLVAL**	**KEIRLEHEEG**	**APCTAIREVS**	240
241	**LLKDLKHANI**	**VTLHDIVHTD**	**KSLTLVFEYL**	**DKDLKQYMDD**	**CGNIMSMHNV**	**KLFLYQILRG**	300
301	**LAYCHRRKVL**	**HRDLKPQNLL**	**INEKGELKLA**	**DFGLARAKSV**	**PTKTYSNEVV**	**TLWYRPPDVL**	360
361	**LGSSEYSTQI**	**DMWGVGCIFF**	**EMASGRPLFP**	**GSTVEDELHL**	**IFRLLGTPSQ**	**ETWPGISSNE**	420
421	**EFKNYNFPKY**	**KPQPLINHAP**	**RLDSEGIELI**	**TKFLQYESKK**	**RVSAEEAMKH**	**VYFRSLGPRI**	480
481	**HALPESVSIF**	**SLKEIQLQKD**	**PGFRNSSYPE**	**TGHGKNRRQS**	**MLF**		540

blue: CDK17 sequence expressed in fusionprotein

[1] NCBI/Protein accession number NP_002586.2

p35NCK 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	PMG**HHHHHH**G	RRRASVAAGI	240
241	**LVPRGS**PGLD	GIYARGIQAS	**MGTVLSLSPS**	**YRKATLFEDG**	**AATVGHYTAV**	**QNSKNAKDKN**	300
301	**LKRHSIISVL**	**PWKRIVAVSA**	**KKKNSKKVQP**	**NSSYQNNITH**	**LNNENLKKSL**	**SCANLSTFAQ**	360
361	**PPPAQPPAPP**	**ASQLSGSQTG**	**GSSSVKKAPH**	**PAVTSAGTPK**	**RVIVQASTSE**	**LLRCLGEFLC**	420
421	**RRCYRLKHLS**	**PTDPVLWLRS**	**VDRSLLLQGW**	**QDKGFITPAN**	**VVFLYMLCRD**	**VISSEVGSDH**	480
481	**ELQAVLLTCL**	**YLSYSYMGNE**	**ISYPLKPFLV**	**ESCKEAFWDR**	**CLSVINLMSS**	**KMLQINADPH**	540
541	**YFTQVFSDLK**	**NESGQEDKKR**	**LLLGLDR**				600

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin **blue**: p35NCK **boxed**:variation from RefSeq

p35NCK wt[2] Amino Acid Sequence

1	**MGTVLSLSPS**	**YRKATLFEDG**	**AATVGHYTAV**	**QNSKNAKDKN**	**LKRHSIISVL**	**PWKRIVAVSA**	60
61	**KKKNSKKVQP**	**NSSYQNNITH**	**LNNENLKKSL**	**SCANLSTFAQ**	**PPPAQPPAPP**	**ASQLSGSQTG**	120
121	**GSSSVKKAPH**	**PAVTSAGTPK**	**RVIVQASTSE**	**LLRCLGEFLC**	**RRCYRLKHLS**	**PTDPVLWLRS**	180
181	**VDRSLLLQGW**	**QDQGFITPAN**	**VVFLYMLCRD**	**VISSEVGSDH**	**ELQAVLLTCL**	**YLSYSYMGNE**	240
241	**ISYPLKPFLV**	**ESCKEAFWDR**	**CLSVINLMSS**	**KMLQINADPH**	**YFTQVFSDLK**	**NESGQEDKKR**	300
301	**LLLGLDR**						360

blue: p35NCK sequence expressed in fusionprotein **Red**: variant in fusionprotein

[2] NCBI/Protein accession number NP_003876.1
Q193K: SNP variation see NCBI/dbSNP ID: rs17852832