

## ProQinase™ CDK5/p35NCK

cyclin dependent kinase 5

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK5

Synonyms: PSSALRE

Product No.: 0356-0355-1

Lot: 001

**Description:** Human CDK5, full length, amino acids M<sub>1</sub>-P<sub>292</sub> (as in NCBI/Protein entry NP\_004926.1) and human p35NCK, amino acids M<sub>1</sub>-R<sub>307</sub> (as in NCBI/Protein entry NP\_003876.1), both N-terminally fused to GST-Thrombin cleavage site, co-expressed in Sf9 insect cells

**Product identity:** CDK5/p35NCK Lot 001, was confirmed as CDK5/p35NCK by specific Western Blotting using anti CDK5 and p35NCK antibodies

**Theoretical MW<sub>CDK5</sub>:** 62,841 Da

**Theoretical MW<sub>p35NCK</sub>:** 63,956 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** GST-Affinity Chromatography

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

**Storage buffer:** 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 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:** 0.240 µg/µl

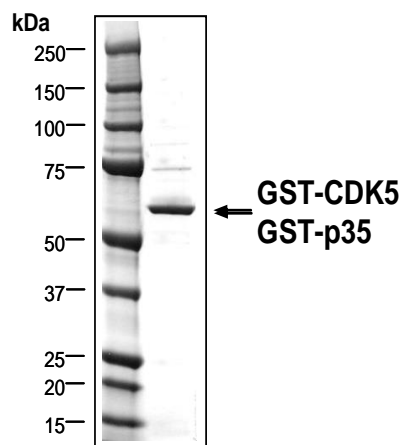
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

Specific kinase activity (P<sub>i</sub> transfer): 11 pmol/µg×min

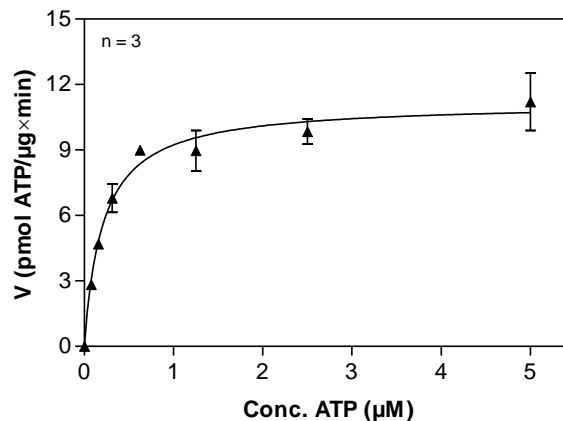
ATP-K<sub>M</sub>: 0.2 µM

CDK5/p35NCK Lot 001:  
Coomassie stain



2.0 µg GST-CDK5/p35

CDK5/p35NCK Lot 001:  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



**Determination of K<sub>M</sub> value & Specific activity:**

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: RB-CTF, 50 µg/ml
  - CDK5/p35NCK: 1.0 µg/ml
- Filter binding assay
  - MSFC membrane (Millipore)

**Additional assay technology:** CDK5/p35NCK Lot 001

was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



# ProQinase™ CDK5/p35NCK

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GST-CDK5 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARPMQKY	EKLEKIGEGT	YGTVFKAKNR	ETHEIVALKR	VRLDDDDEGV	300
301	PSSALREICL	LKELKHKNIV	RLHDVLHSDK	KLTLVFEFCD	QDLKDYFDSC	NGDLDPEIVK	360
361	SFLFQLLKGL	GFCHSRNVLH	RDLKPQNLLI	NRNGELKLAD	FGLARAFGIP	VRCYSAEVVT	420
421	LWYRPPDVLV	GAKLYSTSID	MWSAGCIFAE	LANAGRPLFP	GNDVDDQLKR	IFRLLGTPTE	480
481	EQWPSMTKLP	DYKPYMPYPA	TTSLVNVVVK	LNATGRDLLQ	NLLKCNPVQR	ISAEALQHP	540
541	YFSDFCPP						600

1-218: GST Red: HIS6-tag Pink: Thrombin cleavage site blue: CDK5

CDK5 wt <sup>1</sup> Amino Acid Sequence							
1	MQYKYELEKI	GEGTYGTVFK	AKNRETIEIV	ALKRVRLDDD	DEGVPSSALR	EICLLKELKH	60
61	KNIVRLHDVL	HSDKKLTLVF	EFCDQDLKDY	FDSCNGDLDP	EIVKSFLFQL	LKGLGFCHSR	120
121	NVLHRDLKPQ	NLLINRNGEL	KLADFGFLARA	FGIPVRCYSA	EVVTLWYRPP	DVLFGAKLYS	180
181	TSIDMWSAGC	IFAEANAGR	PLFPGNDVDD	QLKRIFRLLG	TPTEEQWPSM	TKLPDYKPYP	240
241	MYPATTSLVN	VVPKLNATGR	DLLQNLKCN	PVQRISAEEA	LQHPYFSDFC	PP	300

blue: CDK5 sequence expressed in fusionprotein

<sup>1</sup>NCBI/Protein accession number NP\_004926.1

GST-p35NCK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQAS	MGTVLSLSPS	YRKATLFEDG	AATVGHYTAV	QNSKNAKDKN	300
301	LKRHSIISVL	PWKRIVAVSA	KKKNSKKVQP	NSSYQNNITH	LNNENLKKS	SCANLSTFAQ	360
361	PPPAQPPAPP	ASQLSGSQTG	GSSSVKKAPH	PAVTSAGTPK	RVIVQASTSE	LLRCLGEFLC	420
421	RRCYRLKHL	PTDPVLWLR	VDRSLLQGW	QDKGFITPAN	VVFLYMLCRD	VISSEVSDH	480
481	ELQAVLLTCL	YLSYSYMGNE	ISYPLKPFV	ESCKEAFWDR	CLSVINLMSS	KMLQINADPH	540
541	YFTQVFSDLK	NESGQEDKKR	LLLGLDR				600

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

p35NCK wt <sup>1</sup> amino acid sequence							
1	MGTVLSLSPS	YRKATLFEDG	AATVGHYTAV	QNSKNAKDKN	LKRHSIISVL	PWKRIVAVSA	60
61	KKKNSKKVQP	NSSYQNNITH	LNNENLKKS	SCANLSTFAQ	PPPAQPPAPP	ASQLSGSQTG	120
121	GSSSVKKAPH	PAVTSAGTPK	RVIVQASTSE	LLRCLGEFLC	RRCYRLKHL	PTDPVLWLR	180
181	VDRSLLQGW	QDKGFITPAN	VVFLYMLCRD	VISSEVSDH	ELQAVLLTCL	YLSYSYMGNE	240
241	ISYPLKPFV	ESCKEAFWDR	CLSVINLMSS	KMLQINADPH	YFTQVFSDLK	NESGQEDKKR	300
301	LLLGLDR						360

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

<sup>1</sup>NCBI/Protein accession number NP\_003876.1

HGNC identifier: CDK5R1

Q193K: SNP variation see NCBI/dbSNP ID: rs17852832