

Certificate of Analysis

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 M₁-P₂₉₂ (as in NCBI/Protein entry NP_004926.1) and human p35NCK, amino acids M₁-R₃₀₇ (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_{CDK5}: 62,841 Da

Theoretical MW_{p35NCK}: 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

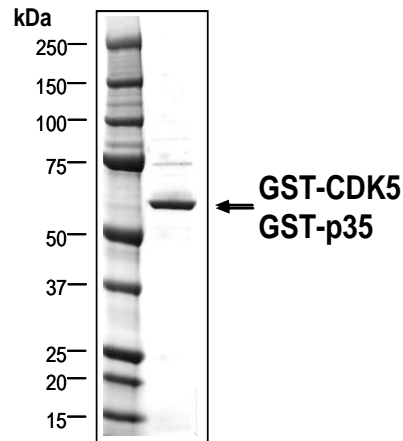
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 11 pmol/µg×min

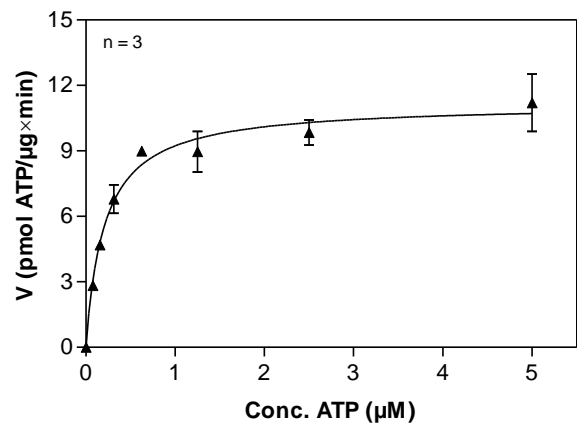
ATP-K_M: 0.2 µM

**CDK5/p35NCK Lot 001:
Coomassie stain**



2.0 µg GST-CDK5/p35

**CDK5/p35NCK Lot 001:
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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - 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 ProQinase for the use with the ADP-Glo™ Kinase assay from Promega



CDK5/p35NCK

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GST-CDK5 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH G	RRRASVAAGI	240
241	LVPRGS PGLD	GIYAR PMQKY	EKLEKIGEGT	YGTVFKAKNR	ETHEIVALKR	VRLDDDDDEGV	300
301	PSSALREICL	LKELKHKNIV	RLHDVLSHSDK	KLTLVFEFCD	QDLKKYFDSC	NGDLDP EIVK	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 ¹ Amino Acid Sequence							
1	MQKYEKLEKI	GEGTYGTVFK	AKNRETHEIV	ALKRVRLDDD	DEGVPSSALR	EICLLKELKH	60
61	KNIVRLHDVL	HSDKKLTLVF	EFCDQDLKKY	FDSCNGDLDP	EIVKSFLFQL	KLGLGFCHSR	120
121	NVLHRDLKPQ	NLLINRNGEL	KLADFLARA	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

¹NCBI/Protein accession number NP_004926.1

GST-p35NCK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH G	RRRASVAAGI	240
241	LVPRGS PGLD	GIYAR GIQAS	MGTVLSLSPS	YRKATLFEDG	AATVGHYTAV	QNSKNAKDKN	300
301	LKRHSIISVL	PWKRIVAVSA	KKKNSKKVQP	NSSYQNNITH	LNNENLKKSL	SCANLSTFAQ	360
361	PPPAQPPAPP	ASQLSGSQTG	GSSSVK KAPH	PAVTSAGTPK	RVIVQASTSE	LLRCLGEFLC	420
421	RRCYRLKHL	PTDPVLWLR	VDRSLLQGW	QDKGFITPAN	VVFLYMLCRD	VISSEVGS DH	480
481	ELQAVLLTCL	YLSYSYMGNE	ISYPLK PFLV	ESCKEAFWDR	CLSVINLMSS	KMLQINADPH	540
541	YFTQVFS DLK	NESGQEDK KR	LLLGLDR				600

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

p35NCK wt ¹ amino acid sequence							
1	MGTVLSLSPS	YRKATLFEDG	AATVGHYTAV	QNSKNAKDKN	LKRHSIISVL	PWKRIVAVSA	60
61	KKKNSKKVQP	NSSYQNNITH	LNNENLKKSL	SCANLSTFAQ	PPPAQPPAPP	ASQLSGSQTG	120
121	GSSSVK KAPH	PAVTSAGTPK	RVIVQASTSE	LLRCLGEFLC	RRCYRLKHL	PTDPVLWLR	180
181	VDRSLLQGW	QDKGFITPAN	VVFLYMLCRD	VISSEVGS DH	ELQAVLLTCL	YLSYSYMGNE	240
241	ISYPLK PFLV	ESCKEAFWDR	CLSVINLMSS	KMLQINADPH	YFTQVFS DLK	NESGQEDK KR	300
301	LLLGLDR						360

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

¹NCBI/Protein accession number NP_003876.1

HGNC identifier: CDK5R1

Q193K: SNP variation see NCBI/dbSNP ID: rs17852832