

ProQinase™ CDK5/p25NCK

cyclin dependent kinase 5

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK5

Synonyms: PSSALRE

Product No.: 0356-0389-1

Lot: 001

Description: Human CDK5, full length, amino acids M₁-P₂₉₂ ([NCBI/Protein](#) entry NP_004926.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site and human p25NCK (CDK5R1) amino acids A₁₀₄-R₃₀₇ ([NCBI/Protein](#) entry NP_003876.1), N-terminal HIS₆ fusion protein with a Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK5/p25NCK Lot 001, was confirmed as CDK5/p25NCK by specific Western blotting

Theoretical MW_{GST-CDK5}: 62,841 Da

Theoretical MW_{p25NCK}: 28,134 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

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: 0.102 µg/µl

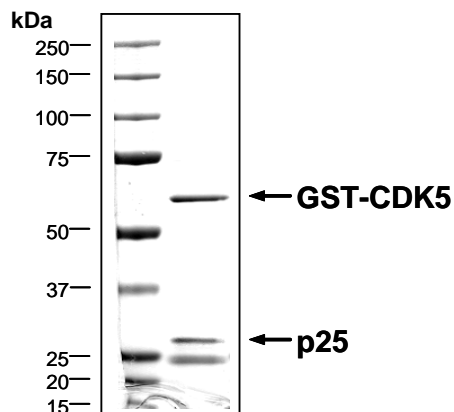
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 16 pmol/µg × min

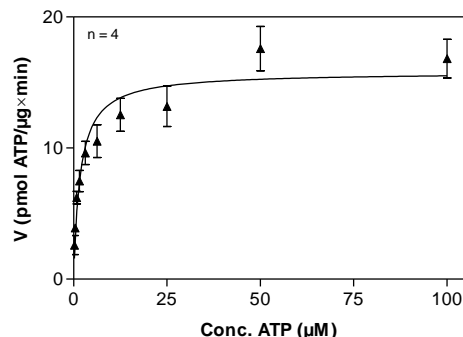
ATP-K_M: 1.8 µM

CDK5/p25NCK Lot 001:
Coomassie stain



2.0 µg GST-CDK5/p25

CDK5/p25NCK 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, 200 µg/ml
 - Kinase: 4 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

ProQinase™ CDK5/p25NCK

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GST-CDK5 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARPMQKY	EKLEKIGEGT	YGTVFKAKNR	ETHEIVALKR	VRLDDDDEGV	300
301	PSSALREICL	LKELKHKNI	RLHDVLHSDK	KLTLVFEFCD	QDLKKYFDCS	NGDLLPEIVK	360
361	SFLFQLLQGL	GFCHSRNVLH	RDLKPQNLLI	NRNGELKLAD	FGLARAFGIP	VRCYSAEVVT	420
421	LWYRPPDVLF	GAKLYSTSID	MWSAGCIFA	LANAGRPLFP	GNDVDDQLKR	IFRLLGTPTE	480
481	EQWPSMTKLP	DYKPYMPYA	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	MQYKLEKI	GEGTYGTVFK	AKNRETHEIV	ALKRVLDDD	DEGVSSALR	EICLLKELKH	60
61	KNIVRLHDVL	HSDKKLTLVF	EFCDDQLKKY	FDSCNGDLDP	EIVKSFLFQL	LKGLGFCHSR	120
121	NVLHRDLKPQ	NLLINRNGEL	KLADFLARA	FGIPVRCYSA	EVVTLWYRPP	DVLFQAKLYS	180
181	TSIDMWSAGC	IFAEANAGR	PLFPNGDVED	QLKRIFRLLG	TPTEEQWPSM	TKLPDYKPY	240
241	MYPATSLVN	VVPKLNATGR	DLQNLKCN	PVQRISAEA	LQHPYFSDFC	PP	300

blue: CDK5 sequence expressed in recombinant protein

¹[NCBI/Protein](#) accession number NP_004926.1

HIS-p25NCK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPIDPMGHH	HHHHGRRRAS	VAAGILVPRG	SPGLDGIYAR	GIQASMAQPP	PAQPPAPPAS	60
61	QLSGSQTGGS	SSVKKAPHPA	VTSAGTPKRV	IVQASTSELL	RCLGEFLCRR	CYRLKHLSP	120
121	DPVLWLRSD	RSLLQGWQD	KGFITPANVV	FLYMLCRDVI	SSEVGSDEL	QAVLLTCLYL	180
181	SYSYMGNEIS	YPLKPFVLES	CKEAFWDRCL	SVINLMSSKM	LQINADPHYF	TQVFSDLKNE	240
241	SGQEDKKRL	LGLDR					300

Red: HIS6-tag Pink: Thrombin cleavage site blue: p25NCK boxed: variation from RefSeq

CDK5R1/p25NCK [#] wt ² Amino Acid Sequence							
1	MGTVLSLSPS	YRKATLFEDG	AATVGHYTAV	QNSKNAKDN	LKRHSIISVL	PWKRIVAVSA	60
61	KKKNSKKVQP	NSSYQNNITH	LNNENLKKSL	SCANLSTFAQ	PPPAQPPAPP	ASQLSGSQTG	120
121	GSSSVKKAPH	PAVTSAGTPK	RVIVQASTSE	LLRCLGEFLC	RRCYRLKHL	PTDPLWLR	180
181	VDRSLLQGW	QDQGFITPAN	VVFLYMLCRD	VISSEVGS	ELQAVLLTCL	YLSYSYMGNE	240
241	ISYPLKPFV	ESCKEAFWDR	CLSVINLMSS	KMLQINADPH	YFTQVFSDLK	NESGQEDKKR	300
301	LLLGLDR						360

blue: p25NCK sequence expressed in recombinant protein Red: variant in recombinant protein

²[NCBI/Protein](#) accession number NP_003876.1

Q193K: SNP variation see [NCBI/dbSNP](#) ID: rs17852832

HGNC identifier p25NCK: CDK5R1

#: p25NCK is generated by proteolytic cleavage of CDK5R1 (p35NCK) by calpain and is not a discrete gene product