

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active CaMK1D [1 – 385]

Enzyme description:- CaMK1D [1 - 385]

Clone number:- DU 37123

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 69,693.33 daltons

Average Mass 69,737.96 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.25

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1 mM CaCl₂, 1 μM Calmodulin

Substrate:-

YLRRRLSDSNF Final concentration: 300 μM

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Clone Data Sheet

CaMK1D [1 – 385]

<u>Protein</u>	CaMK1D [1 - 385]
<u>Clone number</u>	DU 37123
<u>Species</u>	Human
<u>Accession number</u>	NM_153498.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMARENGESSWKK QAEDIKKIFEFKETLGTGAFSEVFLAEEKATGKLFAVKCIPKKALKGKE SSIENEIAVLRKIKHENIVALEDIYESPNHLYLVMQLVSGGELFDRIVE KGFYTEKDASTLIRQVLDAVYYLHRMGIVHRDLKPENLLYYSQDEESKI MISDFGLSKMEGKGDVMSTACGTPGYVAPEVLAQKPYSKAVDCWSIGVI AYILLCGYPPFYDENDSKLFEQILKAIEYEFDSFYWDDISDSAKDFIRNL MEKDPNKRYTCEQAARHPWIAGDTALNKNIHESVSAQIRKNFAKSKWRQ AFNATAVVRHMRKLHLGSSLDSSNASVSSSLSLASQKDCLAPSTLCSFI SSSSGVSGVGAERRPRPTTVTAVHSGSK
<u>Native sequence</u>	Amino acids M1 – K385 (end) of human CaMK1D. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX 6P-1

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Nucleotide

Sequence of insert

ggatccATGGCCCGGGAGAACGGCGAGAGCAGCTCCTCCTGGAAAAAGC
AAGCTGAAGACATCAAGAAGATCTTCGAGTTCAAAGAGACCCTCGGAAC
CGGGGCCTTTTCCGAAGTGGTTTTAGCTGAAGAGAAGGCAACTGGCAAG
CTCTTTGCTGTGAAGTGTATCCCTAAGAAGGCGCTGAAGGGCAAGGAAA
GCAGCATAGAGAAATGAGATAGCCGTCCTGAGAAAAGATTAAGCATGAAAA
TATTGTTGCCCTGGAAGACATTTATGAAAGCCCAAATCACCTGTACTTG
GTCATGCAGCTGGTGTCCGGTGGAGAGCTGTTTGACCGGATAGTGGAGA
AGGGGTTTTATACAGAGAAGGATGCCAGCACTCTGATCCGCCAAGTCTT
GGACGCCGTGTACTATCTCCACAGAATGGGCATCGTCCACAGAGACCTC
AAGCCCGAAAATCTCTTGTAAGTCAAGATGAGGAGTCCAAAATAA
TGATCAGTGACTTTGGATTGTCAAAAATGGAGGGCAAAGGAGATGTGAT
GTCCACTGCCTGTGGAAGTCCAGGCTATGTCGCTCCTGAAGTCCTCGCC
CAGAAACCTTACAGCAAAGCCGTTGACTGCTGGTCCATCGGAGTGATTG
CCTACATCTTGCTCTGCGGCTACCCTCCTTTTTATGATGAAAATGACTC
CAAGCTCTTTGAGCAGATCCTCAAGGCGGAATATGAGTTTGACTCTCCC
TACTGGGATGACATCTCCGACTCTGCAAAAGACTTCATTTCGGAACCTGA
TGGAGAAGGACCCGAATAAAAAGATACACGTGTGAGCAGGCAGCTCGGCA
CCCATGGATCGCTGGTGACACAGCCCTCAACAAAAACATCCACGAGTCC
GTCAGCGCCAGATCCGGAAAAACTTTGCCAAGAGCAAATGGAGACAAG
CATTTAATGCCACGGCCGTCGTGAGACATATGAGAAAACTACACCTCGG
CAGCAGCCTGGACAGTTCAAATGCAAGTGTTCGAGCAGCCTCAGTTTG
GCCAGCCAAAAAGACTGTCTGGCACCTTCCACGCTCTGTAGTTTCATTT
CTTCTTCGTCGGGGGTCTCAGGAGTTGGAGCCGAGCGGAGACCCAGGCC
CACCCTGTGACGGCAGTGCACTCTGGAAGCAAGtgagcggccgc