Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CaMK1B [1 - 426]

Enzyme description:- CaMK1B [1 – 426]

Clone number:- DU 61079

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 74, 107.74 daltons
Average Mass 74, 154.91 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.29

Purity:- >80 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2 -mercaptoethanol, 10 mM magnesium acetate, 0.1 mM CaCl2, 1 μM Calmodulin

Substrate:-
KKALRRQETVDAL Final concentration: 300 μM

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Clone Data Sheet

CaMK1B [1 – 426]

Protein	CaMK1B [1 – 426]
Clone number	DU 61079
Species	Human
Accession number	NM_001039582.3
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MEAAFG** **QVAGSACPRRGGEGRDWKAESLADLWPKSSPGDSHRWCKGPGAGPAGP** **QLREAARASSGLGGGGRHPSRIPAIALQDMLLLKKHTEDISSVYEIRE** **RLGSGAFSEVVLAQERGSAHLVALKCIPKKALRGKEALVENEIAVLRR** **ISHPNIVALEDVHESPSHLYLAMELVTGGELFDRIMERGSYTEKDASH** **LVGQVLGAVSYLHSLGIVHRDLKPENLLYATPFEDSKIMVSDFGLSKI** **QAGNMLGTACGTPGYVAPELLEQKPYGKAVDVWALGVISYILLCGYPP** **FYDESDPELFSQILRASYEFDSPFWDDISESAKDFIRHLLERDPQKRF** **TCQQALRHLWISGDTAFDRDILGSVSEQIRKNFARTHWKRAFNATSFL** **RHIRKLGQIPEGEGASEQGMARHSHSGLRAGQPPKW**
Native sequence	Amino acids M1 – W426 (end) of human CaMK1B. Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Eco*R1 - *Not*1 sites of pGEX6P-1

Nucleotide sequence of insert

gaattcATGGAGGCTGCTTTCGGGCAAGTGGCCGGGTCAGCCTGTCCT
CGGAGAGGCGGTGAAGGCAGAGACTGGAAGGCAGAGAGCCTTGCCGAC
CTGTGGCCGAAGAGCTCTCCGGGAGACAGCCACCGGTGGTGCAAAGGC
CCTGGGGCCGGCCCAGCCGGGCCGCAGCTCCGGGAGGCGGCGCGAGCG
AGCAGTGGGCTGGGCGGTGGCGGCCGGCACCCGAGCCGGATCCCGGCG
ATTGCCTTACAAGACATGCTGCTGCTGAAGAAACACACGGAGGACATC
AGCAGCGTCTACGAGATCCGCGAGAGGCTCGGCTCGGGTGCCTTCTCC
GAGGTGGTGCTGGCCCAGGAGCGGGGCTCCGCACACCTCGTGGCCCTC
AAGTGCATCCCCAAGAAGGCCCTCCGGGGCAAGGAGGCCCTGGTGGAG
AACGAGATCGCAGTGCTCCGTAGGATCAGTCACCCCAACATCGTCGCT
CTGGAGGATGTCCACGAGAGCCCTTCCCACCTCTACCTGGCCATGGAA
CTGGTGACGGGTGGCGAGCTGTTTGACCGCATCATGGAGCGCGGCTCC
TACACAGAGAAGGATGCCAGCCATCTGGTGGGTCAGGTCCTTGGCGCC
GTCTCCTACCTGCACAGCCTGGGGATCGTGCACCGGGACCTCAAGCCC
GAAAACCTCCTGTATGCCACGCCCTTTGAGGACTCGAAGATCATGGTC
TCTGACTTTGGACTCTCCAAAATCCAGGCTGGGAACATGCTAGGCACC
GCCTGTGGGACCCCTGGATATGTGGCCCCAGAGCTCTTGGAGCAGAAA
CCCTACGGGAAGGCCGTAGATGTGTGGGCCCTGGGCGTCATCTCCTAC
ATCCTGCTGTGTGGGTACCCCCCCTTCTACGACGAGAGCGACCCTGAG
CTCTTCAGCCAGATCCTGAGGGCCAGCTATGAGTTTGACTCTCCTTTC
TGGGATGACATCTCAGAATCAGCCAAAGACTTCATCCGGCACCTTCTG
GAGCGAGACCCCCAGAAGAGGTTCACCTGCCAACAGGCCTTGCGGCAC
CTTTGGATCTCTGGGGACACAGCCTTCGACAGGGACATCTTAGGCTCT
GTCAGTGAGCAGATCCGGAAGAACTTTGCTCGGACACACTGGAAGCGA
GCCTTCAATGCCACCTCGTTCCTGCGCCACATCCGGAAGCTGGGGCAG
ATCCCAGAGGGCGAGGGGGCCTCTGAGCAGGGCATGGCCCGCCACAGC
CACTCAGGCCTCCGTGCTGGCCAGCCCCCCAAGTGGtgagcggccgc