Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CaMK1G [1 - 476]

Enzyme description:- CaMK1G [1 – 476]

Clone number:- DU 30189

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 859.39 daltons
Average Mass 79, 911.10 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.75

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

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

Substrate:-
YLRRRLSDSNF Final concentration: 300 μM

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Clone Data Sheet

CaMK1G [1 – 476]

Protein	CaMK1G [1 – 476]
Clone number	DU 30189
Species	Human
Accession number	NM_020439.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MGRKEEDDC** **SSWKKQTTNIRKTFIFMEVLGSGAFSEVFLVKQRLTGKLFALKCIKKS** **PAFRDSSLENEIAVLKKIKHENIVTLEDIYESTTHYYLVMQLVSGGEL** **FDRILERGVYTEKDASLVIQQVLSAVKYLHENGIVHRDLKPENLLYLT** **PEENSKIMITDFGLSKMEQNGIMSTACGTPGYVAPEVLAQKPYSKAVD** **CWSIGVITYILLCGYPPFYEETESKLFEKIKEGYYEFESPFWDDISES** **AKDFICHLLEKDPNERYTCEKALSHPWIDGNTALHRDIYPSVSLQIQK** **NFAKSKWRQAFNAAAVVHHMRKLHMNLHSPGVRPEVENRPPETQASET** **SRPSSPEITITEAPVLDHSVALPALTQLPCQHGRRPTAPGGRSLNCLV** **NGSLHISSSLVPMHQGSLAAGPCGCCSSCLNIGSKGKSSYCSEPTLLK** **KANKKQNFKSEVMVPVKASGSSHCRAGQTGVCLIM**
Native sequence	Amino acids M1 – M476 (end) of human CaMK1G. Residue M232 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	*Bgl*2 - *Not*1 into *Bam*H1- *Not*1 site of pGEX6P-1

Nucleotide sequence of insert

agatctATGGGTCGAAAGGAAGAAGATGACTGCAGTTCCTGGAAGAAA
CAGACCACCAACATCCGGAAAACCTTCATTTTTATGGAAGTGCTGGGA
TCAGGAGCTTTCTCAGAAGTTTTCCTGGTGAAGCAAAGACTGACTGGG
AAGCTCTTTGCTCTGAAGTGCATCAAGAAGTCACCTGCCTTCCGGGAC
AGCAGCCTGGAGAATGAGATTGCTGTGTTGAAAAAGATCAAGCATGAA
AACATTGTGACCCTGGAGGACATCTATGAGAGCACCACCCACTACTAC
CTGGTCATGCAGCTTGTTTCTGGTGGGGAGCTCTTTGACCGGATCCTG
GAGCGGGGTGTCTACACAGAGAAGGATGCCAGTCTGGTGATCCAGCAG
GTCTTGTCGGCAGTGAAATACCTACATGAGAATGGCATCGTCCACAGA
GACTTAAAGCCCGAAAACCTGCTTTACCTTACCCCTGAAGAGAACTCT
AAGATCATGATCACTGACTTTGGTCTGTCCAAGATGGAACAGAATGGC
ATCATGTCCACTGCCTGTGGGACCCCAGGCTACGTGGCTCCAGAAGTG
CTGGCCCAGAAACCCTACAGCAAGGCTGTGGATTGCTGGTCCATCGGC
GTCATCACCTACATATTGCTCTGTGGATACCCCCCGTTCTATGAAGAA
ACGGAGTCTAAGCTTTTCGAGAAGATCAAGGAGGGCTACTATGAGTTT
GAGTCTCCATTCTGGGATGACATTTCTGAGTCAGCCAAGGACTTTATT
TGCCACTTGCTTGAGAAGGATCCGAACGAGCGGTACACCTGTGAGAAG
GCCTTGAGTCATCCCTGGATTGACGGAAACACAGCCCTCCACCGGGAC
ATCTACCCATCAGTCAGCCTCCAGATCCAGAAGAACTTTGCTAAGAGC
AAGTGGAGGCAAGCCTTCAACGCAGCAGCTGTGGTGCACCACATGAGG
AAGCTACACATGAACCTGCACAGCCCGGGCGTCCGCCCAGAGGTGGAG
AACAGGCCGCCTGAAACTCAAGCCTCAGAAACCTCTAGACCCAGCTCC
CCTGAGATCACCATCACCGAGGCACCTGTCCTGGACCACAGTGTAGCA
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GCCCCTGGTGGCAGGTCCCTCAACTGCCTGGTCAATGGCTCCCTCCAC
ATCAGCAGCAGCCTGGTGCCCATGCATCAGGGGTCCCTGGCCGCCGGG
CCCTGTGGCTGCTGCTCCAGCTGCCTGAACATTGGGAGCAAAGGAAAG
TCCTCCTACTGCTCTGAGCCCACACTCCTCAAAAAGGCCAACAAAAAA
CAGAACTTCAAGTCGGAGGTCATGGTACCAGTTAAAGCCAGTGGCAGC
TCCCACTGCCGGGCAGGGCAGACTGGAGTCTGTCTCATTATGtgagcg
gccgc