University of Dundee

Standard Operating Procedure

Preparation of active CaMK1 [2 – 369]

Enzyme description:- CaMK1 [2 - 369]

Clone number:- DU 1148

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 8 mg/L

Calculated molecular mass:- 67, 986 daltons

Purity:- 90 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 500 μM $CaCl_2$, 0.3 μM calmodulin, 0.1 mM EGTA,
0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM magnesium acetate

Substrate:-
YLRRRLSDSNF-amide [Residues 3 - 13 of Synapsin 1] Final concentration: 300 μM

Specific activity range:- 800 - 1600 U/mg

University of Dundee

Clone Data Sheet - CaMK1 [2 - 369]

Protein	CaMK1 [2 - 369]
Clone number	DU 1148
Species	Human
Accession number	NM_003656
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**LGAVEGPRWKQAED IRDIYDFRDVLGTGAFSEVILAEDKRTQKLVAIKCIAKEALEGKEGSME NEIAVLHKIKHPNIVALDDIYESGGHLYLIMQLVSGGELFDRIVEKGFY TERDASRLIFQVLDAVKYLHDLGIVHRDLKPENLLYYSLDEDSKIMISD FGLSKMEDPGSVLSTACGTPGYVAPEVLAQKPYSKAVDCWSIGVIAYIL LCGYPPFYDENDAKLFEQILKAEYEFDSPYWDDISDSAKDFIRHLMEKD PEKRFTCEQALQHPWIAGDTALDKNIHQSVSEQIKKNFAKSKWKQAFNA TAVVRHMRKLQLGTSQEGQGQTASHGELLTPVAGGPAAGCCCRDCCVEP GTELSPTLPHQL**
Native sequence	Amino acids L2 – L369 (end) of human CaMK1. Residue L232 of the fusion protein is equivalent to L2 of the native enzyme. The GST tag is located at residues 1 - 220
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX 6P-1

Nucleotide Sequence of insert

GGATCCCTGGGGGCAGTGGAAGGCCCCAGGTGGAAGCAGGCG
GAGGACATTAGAGACATCTACGACTTCCGAGATGTTCTGGGC
ACGGGGGCCTTCTCGGAGGTGATCCTGGCAGAAGATAAGAGG
ACGCAGAAGCTGGTGGCCATCAAATGCATTGCCAAGGAGGCC
CTGGAGGGCAAGGAAGGCAGCATGGAGAATGAGATTGCTGTC
CTGCACAAGATCAAGCACCCCAACATTGTAGCCCTGGATGAC
ATCTATGAGAGTGGGGGCCACCTCTACCTCATCATGCAGCTG
GTGTCGGGTGGGGAGCTCTTTGACCGTATTGTGGAAAAAGGC
TTCTACACGGAGCGGGACGCCAGCCGCCTCATCTTCCAGGTG
CTGGATGCTGTGAAATACCTGCATGACCTGGGCATTGTACAC
CGGGATCTCAAGCCAGAGAATCTGCTGTACTACAGCCTGGAT
GAAGACTCCAAAATCATGATCTCCGACTTTGGCCTCTCCAAG
ATGGAGGACCCGGGCAGTGTGCTCTCCACCGCCTGTGGAACT
CCGGGATACGTGGCCCCTGAAGTCCTGGCCCAGAAGCCCTAC
AGCAAGGCTGTGGATTGCTGGTCCATAGGTGTCATCGCCTAC
ATCTTGCTCTGCGGTTACCCTCCCTTCTATGACGAGAATGAT
GCCAAACTCTTTGAACAGATTTTGAAGGCCGAGTACGAGTTT
GACTCTCCTTACTGGGACGACATCTCTGACTCTGCCAAAGAT
TTCATCCGGCACTTGATGGAGAAGGACCCAGAGAAAAGATTC
ACCTGTGAGCAGGCCTTGCAGCACCCATGGATTGCAGGAGAT
ACAGCTCTAGATAAGAATATCCACCAGTCGGTGAGTGAGCAG
ATCAAGAAGAACTTTGCCAAGAGCAAGTGGAAGCAAGCCTTC
AATGCCACGGCTGTGGTGCGGCACATGAGGAAACTGCAGCTG
GGCACCAGCCAGGAGGGGCAGGGGCAGACGGCGAGCCATGGG
GAGCTGCTGACACCAGTGGCTGGGGGGCCGGCAGCTGGCTGT
TGCTGTCGAGACTGCTGCGTGGAGCCGGGCACAGAACTGTCC
CCCACACTGCCCCACCAGCTCtaggaattc