University of Dundee

Standard Operating Procedure

Preparation of active CaMKK beta isoform 2 [1 – 541]

Enzyme description:- CaMKK beta isoform 2 [1 - 541]

Clone number:- DU 8964

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 86, 370.75 daltons
Average Mass 86, 425.97 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.59

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:- 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, 10 mM magnesium acetate

Substrate:-

DGEFLRTSCGSPNYAARRR	residues 168 – 183 of human AMPK1
(plus added Arg residues at C terminus)	Final concentration: 300 μM

Specific activity range:- To be determined

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Clone Data Sheet

CaMKK beta isoform 2 [1 - 541]

Protein	CaMKK beta isoform 2 [1 - 541]
Clone number	DU 8964
Species	Human
Accession number	NM_153499.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSSCVSSQPSSNRA APQDELGGRGSSSSESQKPCEALRGLSSLSIHLGMESFIVVTECEPGCA VDLGLARDRPLEADGQEVPLDTSGSQARPHLSGRKLSLQERSQGGLAAG GSLDMNGRCICPSLPYSPVSSPQSSPRLPRRPTVESHHVSITGMQDCVQ LNQYTLKDEIGKGSYGVVKLAYNENDNTYYAMKVLSKKKLIRQAGFPRR PPPRGTRPAPGGCIQPRGPIEQVYQEIAILKKLDHPNVVKLVEVLDDPN EDHLYMVFELVNQGPVMEVPTLKPLSEDQARFYFQDLIKGIEYLHYQKI IHRDIKPSNLLVGEDGHIKIADFGVSNEFKGSDALLSNTVGTPAFMAPE SLSETRKIFSGKALDVWAMGVTLYCFVFGQCPFMDERIMCLHSKIKSQA LEFPDQPDIAEDLKDLITRMLDKNPESRIVVPEIKLHPWVTRHGAEPLP SEDENCTLVEVTEEEVENSVKHIPSLATVILVKTMIRKRSFGNPFEGSR REERSLSAPGNLLTKQGSEDNLQGTDPPPVGEEEVLL**
Native sequence	Amino acids M1 – L541 (end) of human CaMKK beta isoform 2. 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 (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1site of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGTCATCATGTGTCTCTAGCCAGCCCAGCAGCAACCGGGCCG
CCCCCCAGGATGAGCTGGGGGGCAGGGGCAGCAGCAGCAGCGAAAGCCA
GAAGCCCTGTGAGGCCCTGCGGGGCCTCTCATCCTTGAGCATCCACCTG
GGCATGGAGTCCTTCATTGTGGTCACCGAGTGTGAGCCGGGCTGTGCTG
TGGACCTCGGCTTGGCGCGGGACCGGCCCCTGGAGGCCGATGGCCAAGA
GGTCCCCCTTGACACCTCCGGGTCCCAGGCCCGGCCCCACCTCTCCGGT
CGCAAGCTGTCTCTGCAAGAGCGGTCCCAGGGTGGGCTGGCAGCCGGTG
GCAGCCTGGACATGAACGGACGCTGCATCTGCCCGTCCCTGCCCTACTC
ACCCGTCAGCTCCCCGCAGTCCTCGCCTCGGCTGCCCCGGCGGCCGACA
GTGGAGTCTCACCACGTCTCCATCACGGGTATGCAGGACTGTGTGCAGC
TGAATCAGTATACCCTGAAGGATGAAATTGGAAAGGGCTCCTATGGTGT
CGTCAAGTTGGCCTACAATGAAAATGACAATACCTACTATGCAATGAAG
GTGCTGTCCAAAAAGAAGCTGATCCGGCAGGCCGGCTTTCCACGTCGCC
CTCCACCCCGAGGCACCCGGCCAGCTCCTGGAGGCTGCATCCAGCCCAG
GGGCCCCATTGAGCAGGTGTACCAGGAAATTGCCATCCTCAAGAAGCTG
GACCACCCCAATGTGGTGAAGCTGGTGGAGGTCCTGGATGACCCCAATG
AGGACCATCTGTACATGGTGTTCGAACTGGTCAACCAAGGGCCCGTGAT
GGAAGTGCCCACCCTCAAACCACTCTCTGAAGACCAGGCCCGTTTCTAC
TTCCAGGATCTGATCAAAGGCATCGAGTACTTACACTACCAGAAGATCA
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CATCAAGATCGCTGACTTTGGTGTGAGCAATGAATTCAAGGGCAGTGAC
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TGGAATTTCCAGACCAGCCCGACATAGCTGAGGACTTGAAGGACCTGAT
CACCCGTATGCTGGACAAGAACCCCGAGTCGAGGATCGTGGTGCCGGAA
ATCAAGCTGCACCCCTGGGTCACGAGGCATGGGGCGGAGCCGTTGCCGT
CGGAGGATGAGAACTGCACGCTGGTCGAAGTGACTGAAGAGGAGGTCGA
GAACTCAGTCAAACACATTCCCAGCTTGGCAACCGTGATCCTGGTGAAG
ACCATGATACGTAAACGCTCCTTTGGGAACCCATTCGAGGGCAGCCGGC
GGGAGGAACGCTCACTGTCAGCGCCTGGAAACTTGCTCACGAAGCAAGG
CAGCGAAGACAACCTCCAGGGCACCGACCCGCCCCCCGTGGGGGAGGAG
GAAGTGCTCTTGtgagc