

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

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

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

**Clone number:-** DU 8205

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Expression level:-** 2 mg/L

**Calculated molecular mass:-**

Monoisotopic 88,020.47 daltons

Average Mass 88,076.88 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.64

**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:-** -20 °C

**Assay:-** Standard filter binding assay

**Assay buffer:-**

50 mM Tris-HCl pH 7.5, 500  $\mu$ M CaCl<sub>2</sub>, 0.3  $\mu$ M calmodulin, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate

**Substrate:-**

AKPKGKDYHLQTCCGSLAYRRR, residues 155 – 175 of human MELK (T loop + added Arg residues at C terminus)

Final concentration: 300  $\mu$ M

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**Specific activity range:-** To be determined

### **Clone Data Sheet**

#### **CaMKK beta isoform 2 [1 - 541]**

**Protein** CaMKK beta isoform 2 [1 - 541]

**Clone number** DU 8205

**Species** Human

**Accession number** NM\_153499

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLG SMSNGRQCAGIRPCE  
**FMSSCVSSQPSSNRAAPQDELGGRGSSSESQKPCEALRGLSSLSIHLG**  
**MESFIVVTECEPGCAVDLGLARDRPLEADGQEVPLDTSGSQARPHLSGR**  
**KLSQLQERSQGGLAAGGSLDMNGRCICPSLPYSPVSSPQSSPRLPRRPTV**  
**ESHV SITGMQDCVQLNQYTLKDEIGKGSYGVVKLAYNENDNTYYAMKV**  
**LSKKKLI RQAGFPRRPPRGTRPAPGGCIQPRGPIEQVYQEIAILKKLD**  
**HPNVVKLVEVLDDPNEDHLYMV FELVNQGPVMEVPTLKPLSEDQARFYF**  
**QDLIKGIEYLHYQKI IHRDIKPSNLLVGEDGHIKIADFGVSNEFKGSDA**  
**LLSNTVGT PAFMAPESLSETRKIFSGKALDVWAMGVTLYCFVFGQCPFM**  
**DERIMCLHSKIKSQALEFPDQPDIAEDLKDLITRMLDKNPESRIVVPEI**  
**KLHPWVTRHGAEPLPSEENCTLVEVTEEEVENSVKHIPSLATVILVKT**  
**MIRKRSFGNPFEGSRREERSLSAPGNLLTKQGEDNLQGTDP PPVGEET**  
**VLL**

**Native sequence** Amino acids M1 – L541 (end) of human CaMKK beta isoform 2.  
Residue M247 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*I site of pGEX 6P-1

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**Nucleotide**  
**Sequence of insert**

ggatccatgagtaacggccgcccagtggtgctggaattcgcccttgcaat  
tcATGTCATCATGTGTCTCTAGCCAGCCCAGCAGCAACCGGGCCGCCCC  
CCAGGATGAGCTGGGGGGCAGGGGCAGCAGCAGCAGCGAAAGCCAGAAG  
CCCTGTGAGGCCCTGCGGGGCCCTCTCATCCTTGAGCATCCACCTGGGCA  
TGGAGTCCTTCATTGTGGTCACCGAGTGTGAGCCGGGCTGTGCTGTGGA  
CCTCGGCTTGGCGCGGGACCGGCCCTGGAGGCCGATGGCCAAGAGGTC  
CCCCTTGACACTTCCGGGTCCCAGGCCCGGCCACCTCTCCGGTCGCA  
AGCTGTCTCTGCAAGAGCGGTCCCAGGGTGGGCTGGCAGCCGGTGGCAG  
CCTGGACATGAACGGACGCTGCATCTGCCCGTCCCTGCCCTACTCACCC  
GTCAGCTCCCCGCAGTCCCTCGCCTCGGCTGCCCGGGCGGCCGACAGTGG  
AGTCTCACCACGTCTCCATCACGGGTATGCAGGACTGTGTGCAGCTGAA  
TCAGTATAACCCTGAAGGATGAAATTGGAAAGGGCTCCTATGGTGTGTC  
AAGTTGGCCTACAATGAAAATGACAATACCTACTATGCAATGAAGGTGC  
TGTCCAAAAGAAGCTGATCCGGCAGGCCGGCTTTCCACGTGCCCCCTCC  
ACCCCGAGGCACCCGGCCAGCTCCTGGAGGCTGCATCCAGCCCAGGGGC  
CCCATTGAGCAGGTGTACCAGGAAATTGCCATCCTCAAGAAGCTGGACC  
ACCCCAATGTGGTGAAGCTGGTGGAGGTCCTGGATGACCCCAATGAGGA  
CCATCTGTACATGGTGTTCGAACTGGTCAACCAAGGGCCCGTGATGGAA  
GTGCCACCCTCAAACCACTCTCTGAAGACCAGGCCCGTTTTCTACTTCC  
AGGATCTGATCAAAGGCATCGAGTACTTACACTACCAGAAGATCATCCA  
CCGTGACATCAAACCTTCCAACCTCCTGGTCCGAGAAGATGGGCACATC  
AAGATCGCTGACTTTGGTGTGAGCAATGAATTCAGGGCAGTGACGCGC  
TCCTCTCCAACACCGTGGGCACGCCCGCCTTCATGGCACCCGAGTCGCT  
CTCTGAGACCCGCAAGATCTTCTCTGGGAAGGCCCTTGGATGTTTGGGCC  
ATGGGTGTGACACTATACTGCTTTGTCTTTGGCCAGTGCCCATTCATGG  
ACGAGCGGATCATGTGTTTACACAGTAAGATCAAGAGTCAGGCCCTGGA  
ATTTCCAGACCAGCCCGACATAGCTGAGGACTTGAAGGACCTGATCACC  
CGTATGCTGGACAAGAACCCCGAGTCGAGGATCGTGGTGCCGGAATCA  
AGCTGCACCCCTGGGTCACGAGGCATGGGGCGGAGCCGTTGCCGTCGGA  
GGATGAGAACTGCACGCTGGTTCGAAGTGAAGAGGAGGTGAGAAC  
TCAGTCAAACACATTCCCAGCTTGGCAACCGTGATCCTGGTGAAGACCA  
TGATACGTAAACGCTCCTTTGGGAACCCATTCGAGGGCAGCCGGCGGGA  
GGAACGCTCACTGTCAGCGCCTGAAACTTGCTCACGAAGCAAGGCAGC  
GAAGACAACCTCCAGGGCACCGACCCGCCCCCGTGGGGGAGGAGGAAG  
TGCTCTTgtgagaggcagtcacctgcgtggaaagtgtgctgggcccccgcc  
ccccgctccccgcacgcatgcatccactgcggccggaggagccatgg  
agcccgagtagcatttcatcatcaccatcaccattgagcggccgc