Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CaMKK alpha isoform a [1 – 505]

Enzyme description:- CaMKK alpha isoform a [1 - 505]

Clone number:- DU 8208

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and C-teminal His(6)

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 83, 686.83 daltons
Average Mass 83, 739.84 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.75

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 μM $CaCl_2$, 0.3 μM calmodulin, 0.1 mM EGTA,
0.1 % 2–mercaptoethanol, 10 mM magnesium acetate

Substrate:-
AKPKGNKDYHLQTCCGSLAYRRR, residues 155 – 175 of human MELK
(T loop + added Arg residues at C terminus)
Final concentration: 300 μM

Specific activity range:- To be determined

Clone Data Sheet

CaMKK alpha isoform a [1 - 505]

Protein	CaMKK alpha isoform a [1 - 505]
Clone number	DU 8208
Species	Human
Accession number	NM_172206
Tags	N-terminal GST and C-terminal His(6)
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MEGGPAVCCQD** **PRAELVERVAAIDVTHLEEADGGPEPTRNGVDPPPRARAASVIPGSTSR** **LLPARPSLSARKLSLQERPAGSYLEAQAGPYATGPASHISPRAWRRPTI** **ESHHVAISDAEDCVQLNQYKLQSEIGKGAYGVVRLAYNESEDRHYAMKV** **LSKKKLLKQYGFPRRPPPRGSQAAQGGPAKQLLPLERVYQEIAILKKLD** **HVNVVKLIEVLDDPAEDNLYLVFDLLRKGPVMEVPSDKPFSEEQARLYL** **RDVILGLEYLHCQKIVHRDIKPSNLLLGDDGHVKIADFGVSNQFEGNDA** **QLSSTAGTPAFMAPEAISDSGQSFSGKALDVWATGVTLYCFVYGKCPFI** **DDFILALHRKIKNEPVVFPEEPEISEELKDLILKMLDKNPETRIGVPDI** **KLHPWVTKNGEEPLPSEEEHCSVVEVTEEEVKNSVRLIPSWTTVILVKS** **MLRKRSFGNPFEPQARREERSMSAPGNLLVKEGFGEGGKSPELPGVQED** **EAAS**HHHHHH
Native sequence	Amino acids M1 – S505 (end) of human CaMKK alpha isoform a. Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220 and the His(6) tag is located at residues 740 – 745.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Eco*R1 and *Not*1site of pGEX 6P-1

Nucleotide Sequence of insert

gaattcATGGAGGGGGGTCCAGCTGTCTGCTGCCAGGATCCTCGGGCAG
AGCTGGTAGAACGGGTGGCAGCCATCGATGTGACTCACTTGGAGGAGGC
AGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCCACCACGG
GCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAG
CCCGGCCTAGCCTCTCAGCCAGGAAGCTTTCCCTACAGGAGCGGCCAGC
AGGAAGCTATCTGGAGGCGCAGGCTGGGCCTTATGCCACGGGGCCTGCC
AGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACC
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GCTGCAGAGTGAGATTGGCAAGGGTGCCTACGGTGTGGTGAGGCTGGCC
TACAACGAAAGTGAAGACAGACACTATGCAATGAAAGTCCTTTCCAAAA
AGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGG
GTCCCAGGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCTGCCCCTGGAG
CGGGTGTACCAGGAGATTGCCATCCTGAAGAAGCTGGACCACGTGAATG
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TTTGGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCAGT
GACAAGCCCTTCTCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCA
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CAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCC
GACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCA
GCACGGCGGGAACCCCAGCATTCATGGCCCCCGAGGCCATTTCTGATTC
CGGCCAGAGCTTCAGTGGGAAGGCCTTGGATGTATGGGCCACTGGCGTC
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ATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAA
GGGGGCAAGAGCCCAGAGCTCCCCGGCGTCCAGGAAGACGAGGCTGCAT
CCCATCATCACCATCACCATtgagcggccgca