Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MARK4 [2 - 752]

Enzyme description:- MARK4 [2 - 752]

Clone number:- DU 1281

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 1 mg/L

Calculated molecular mass:-
Monoisotopic 85, 706.73 daltons
Average Mass 85, 760.13 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.58

Purity:- 75 %

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 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-
CHKtide peptide [KKKVSRSGLYRSPSMPENLNRPR]
Final concentration: 300 μM

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Clone Data Sheet

MARK4 [2 - 752]

Protein	MARK4 [2 – 752]
Clone Number	DU 1281
Species	Human
Accession number	AK075272
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**SSRTVLAPGNDRNSDTHGTLG SGRSSDKGPSWSSRSLGARCRNSIASCPEEQPHVGNYRLLRTIGKGNFA KVKLARHILTGREVAIKIIDKTQLNPSSLQKLFREVRIMKGLNHPNIVK LFEVIETEKTLYLVMEYASAGEVFDYLVSHGRMKEKEARAKFRQIVSAV HYCHQKNIVHRDLKAENLLLDAEANIKIADFGFSNEFTLGSKLDTFCGS PPYAAPELFQGKKYDGPEVDIWSLGVILYTLVSGSLPFDGHNLKELRER VLRGKYRVPFYMSTDCESILRRFLVLNPAKRCTLEQIMKDKWINIGYEG EELKPYTEPEEDFGDTKRIEVMVGMGYTREEIKESLTSQKYNEVTATYL LLGRKTEEGGDRGAPGLALARVRAPSDTTNGTSSSKGTSHSKGQRSSSS TYHRQRRHSDFCGPSPAPLHPKRSPTSTGEAELKEERLPGRKASCSTAG SGSRGLPPSSPMVSSAHNPNKAEIPERRKDSTSTPNNLPPSMMTRRNTY VCTERPGAERPSLLPNGKENSSGTPRVPPASPSSHSLAPPSGERSRLAR GSTIRSTFHGGQVRDRRAGGGGGGVQNGPPASPTLAHEAAPLPAGRPR PTTNLFTKLTSKLTRRVADEPERIGGPEVTSCHLPWDQTETAPRLLRFP WSVKLTSSRPPEALMAALRQATAAARCRCRQPQPFLLACLHGGAGGPEP LSHFEVEVCQLPRPGLRGVLFRRVAGTALAFRTLVTRISNDLEL**
Native sequence	Amino acids S2 – L752 (end) of human MARK4. Residue S29 of fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 site of pFastBAC HTb

Nucleotide sequence of insert

ggatccTCTTCGCGGACGGTGCTGGCCCCGGGCAACGATCGGAACTCGG
ACACGCATGGCACCTTGGGCAGTGGCCGCTCCTCGGACAAAGGCCCGTC
CTGGTCCAGCCGCTCACTGGGTGCCCGTTGCCGGAACTCCATCGCCTCC
TGTCCCGAGGAGCAGCCCCACGTGGGCAACTACCGCCTGCTGAGGACCA
TTGGGAAGGGCAACTTTGCCAAAGTCAAGCTGGCTCGGCACATCCTCAC
TGGTCGGGAGGTTGCCATCAAGATTATCGACAAAACCCAGCTGAATCCC
AGCAGCCTGCAGAAGCTGTTCCGAGAAGTCCGCATCATGAAGGGCCTAA
ACCACCCCAACATCGTGAAGCTCTTTGAGGTGATTGAGACTGAGAAGAC
GCTGTACCTGGTGATGGAGTACGCAAGTGCTGGAGAAGTGTTTGACTAC
CTCGTGTCGCATGGCCGCATGAAGGAGAAGGAAGCTCGAGCCAAGTTCC
GACAGATTGTTTCGGCTGTGCACTATTGTCACCAGAAAAATATTGTACA
CAGGGACCTGAAGGCTGAGAACCTCTTGCTGGATGCCGAGGCCAACATC
AAGATTGCTGACTTTGGCTTCAGCAACGAGTTCACGCTGGGATCGAAGC
TGGACACGTTCTGCGGGAGCCCCCCATATGCCGCCCCGGAGCTGTTTCA
GGGCAAGAAGTACGACGGGCCGGAGGTGGACATCTGGAGCCTGGGAGTC
ATCCTGTACACCCTCGTCAGCGGCTCCCTGCCCTTCGACGGGCACAACC
TCAAGGAGCTGCGGGAGCGAGTACTCAGAGGGAAGTACCGGGTCCCTTT
CTACATGTCAACAGACTGTGAGAGCATCCTGCGGAGATTTTTGGTGCTG
AACCCAGCTAAACGCTGTACTCTCGAGCAAATCATGAAAGACAAATGGA
TCAACATCGGCTATGAGGGTGAGGAGTTGAAGCCATACACAGAGCCCGA
GGAGGACTTCGGGGACACCAAGAGAATTGAGGTGATGGTGGGTATGGGC
TACACACGGGAAGAAATCAAAGAGTCCTTGACCAGCCAGAAGTACAACG
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GACACCACCAACGGAACAAGTTCCAGCAAAGGCACCAGCCACAGCAAAG
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TTTCTGTGGCCCATCCCCTGCACCCCTGCACCCCAAACGCAGCCCGACG
AGCACGGGGGAGGCGGAGCTGAAGGAGGAGCGGCTGCCAGGCCGGAAGG
CGAGCTGCAGCACCGCGGGGAGTGGGAGTCGAGGGCTGCCCCCCTCCAG
CCCCATGGTCAGCAGCGCCCACAACCCCAACAAGGCAGAGATCCCAGAG
CGGCGGAAGGACAGCACGAGCACCCCCAACAACCTCCCTCCTAGCATGA
TGACCCGCAGAAACACCTACGTTTGCACAGAACGCCCGGGGGCTGAGCG
CCCGTCACTGTTGCCAAATGGGAAAGAAAACAGCTCAGGCACCCCACGG
GTGCCCCCTGCCTCCCCCTCCAGTCACAGCCTGGCACCCCCATCAGGGG
AGCGGAGCCGCCTGGCACGCGGTTCCACCATCCGCAGCACCTTCCATGG
TGGCCAGGTCCGGGACCGGCGGGCAGGGGGTGGGGGTGGTGGGGGTGTG
CAGAATGGGCCCCCTGCCTCTCCCACACTGGCCCATGAGGCTGCACCCC
TGCCCGCCGGGCGGCCCCGCCCCACCACCAACCTCTTCACCAAGCTGAC
CTCCAAACTGACCCGAAGGGTCGCAGACGAACCTGAGAGAATCGGGGGA
CCTGAGGTCACAAGTTGCCATCTACCTTGGGATCAAACGGAAACCGCCC
CCCGGCTGCTCCGATTCCCCTGGAGTGTGAAGCTGACCAGCTCGCGCCC
TCCTGAGGCCCTGATGGCAGCTCTGCGCCAGGCCACAGCAGCCGCCCGC
TGCCGCTGCCGCCAGCCACAGCCGTTCCTGCTGGCCTGCCTGCACGGGG
GTGCGGGCGGGCCCGAGCCCCTGTCCCACTTCGAAGTGGAGGTCTGCCA
GCTGCCCCGGCCAGGCTTGCGGGGAGTTCTCTTCCGCCGTGTGGCGGGC
ACCGCCCTGGCCTTCCGCACCCTCGTCACCCGCATCTCCAACGACCTC
GAGCTCtgaggatcc