

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MARK4 [2 - 752]

<u>Enzyme description:-</u>	MARK4 [2 - 752]
<u>Clone number:-</u>	DU 1281
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	1 mg/L
<u>Calculated molecular mass:-</u>	85, 706 daltons
<u>Purity:-</u>	75 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	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.
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay Buffer:-</u>	50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc
<u>Substrate:-</u>	AMARA peptide [AMARAASAAALARRR] Final concentration: 300 µM
<u>Specific activity range:-</u>	To be determined

Division of Signal Transduction Therapy

Clone Data Sheet

MARK4 [2 - 752]

<u>Protein</u>	MARK4 [2 – 752]
<u>Clone Number</u>	DU 1281
<u>Species</u>	Human
<u>Accession number</u>	AK075272
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGSSSRTVLAPGNDNRNSDTHGTLG SGRSSDKGPSWSSRSLGARCRNSIASCPPEEQPHVGNRYLLRTIGKGNFA KVKLARHILTGREVAIKIIDKTQLNPSSLQKLFREVRIMKGLNHPNIVK LFEVIETEKTLYLVMYASAGEVFDYLVSHGRMKEKEARAKFRQIVSAV HYCHQKNIVHRDLKAENLLDAEANIKIADFGFSNEFTLGSKLDTFCGS PPYAAPELFQGGKYDGPVDIWSLGVILYTLVSGSLPFDGHNKELRER VLRGKYRVPFYMSTDCESILRRFLVLNPAKRCTLEQIMKDKWINIGYEG EELKPYTEPEEDFGDTKRIEVMVGMGYTREEIKESLTSQKYNEVTATYL LLGRKTEEGGDRGAPGLALARVRAPSDTTNGTSSSKGTSHSKGQRSSSS TYHRQRRHSDFCGPSPAPLHPKRSPTSTGEAELKEERLPGRKASCSTAG SGSRGLPPSSPMVSSAHNPNAEIPERRKDSTSTPNNLPPSMTRRNTY VCTERPGAERPSSLPNGKENSSGTPRVPPASPSHSLAPPSGERSRLAR GSTIRSTFHGGQVRDRRAGGGGGGGVQNGPPASPTLAHEAAPLPAGRPR PTTNLFTKLTSKLTRRVADEPERIGGPEVTSCHLPWDQTETAPRLLRFP WSVKLTSSRPPEALMAALRQATAAARCRCRQPQPFLACLHGGAGGPEP LSHFEVEVCQLPRPGLRGVLFRRVAGTALAFRTLVTIRISNDLEL
<u>Native sequence</u>	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.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	BamH1 site of pFastBAC HTb

Division of Signal Transduction Therapy

Nucleotide sequence of insert

ggatccTCTTCGCGGACGGTGCTGGCCCCGGGCAACGATCGGAACTCGG
ACACGCATGGCACCTTGGGCAGTGGCCGCTCCTCGGACAAAGGCCCGTC
CTGGTCCAGCCGCTCACTGGGTGCCCGTTGCCGGAACCTCATCGCCTCC
TGTCCCAGGAGCAGCCCCACGTGGGCAACTACCGCCTGCTGAGGACCA
TTGGGAAGGGCAACTTTGCCAAAGTCAAGCTGGCTCGGCACATCCTCAC
TGGTCGGGAGGTTGCCATCAAGATTATCGACAAAACCCAGCTGAATCCC
AGCAGCCTGCAGAAGCTGTTCCGAGAAGTCCGCATCATGAAGGGCCTAA
ACCACCCCAACATCGTGAAGCTCTTTGAGGTGATTGAGACTGAGAAGAC
GCTGTACCTGGTGATGGAGTACGCAAGTGCTGGAGAAGTGTTTGACTAC
CTCGTGTCGCATGGCCGCATGAAGGAGAAGGAAGCTCGAGCCAAGTTCC
GACAGATTGTTTCGGCTGTGCACTATTGTCACCAGAAAAATATTGTACA
CAGGGACCTGAAGGCTGAGAACCTCTTGCTGGATGCCGAGGCCAACATC
AAGATTGCTGACTTTGGCTTCAGCAACGAGTTCACGCTGGGATCGAAGC
TGGACACGTTCTGCGGGAGCCCCCATATGCCGCCCGGAGCTGTTTCA
GGGCAAGAAGTACGACGGGCGGAGGTGGACATCTGGAGCCTGGGAGTC
ATCCTGTACACCCTCGTCAGCGGCTCCCTGCCCTTCGACGGGCACAACC
TCAAGGAGCTGCGGGAGCGAGTACTCAGAGGGAAGTACCGGGTCCCTTT
CTACATGTCAACAGACTGTGAGAGCATCCTGCGGAGATTTTTGGTGCTG
AACCCAGCTAAACGCTGTACTCTCGAGCAAATCATGAAAGACAAATGGA
TCAACATCGGCTATGAGGGTGAGGAGTTGAAGCCATACACAGAGCCCGA
GGAGGACTTCGGGGACACCAAGAGAATTGAGGTGATGGTGGGTATGGGC
TACACACGGGAAGAAATCAAAGAGTCCTTGACCAGCCAGAAGTACAACG
AAGTGACCGCCACCTACCTCCTGCTGGGCAGGAAGACTGAGGAGGGTGG
GGACCGGGGCGCCCCAGGGCTGGCCCTGGCACGGGTGCGGGCGCCCAGC
GACACCACCAACGGAACAAGTTCCAGCAAAGGCACCAGCCACAGCAAAG
GGCAGCGGAGTTCCTCTTCCACCTACCACCGCCAGCGCAGGCATAGCGA
TTTCTGTGGCCCATCCCCTGCACCCCTGCACCCCAAACGCAGCCCGACG
AGCACGGGGGAGGCGGAGCTGAAGGAGGAGCGGCTGCCAGGCCGGAAGG
CGAGCTGCAGCACCGCGGGGAGTGGGAGTCGAGGGCTGCCCCCTCCAG
CCCCATGGTCAGCAGCGCCACAACCCCAACAAGGCAGAGATCCCAGAG
CGGCGGAAGGACAGCACGAGCACCCCAACAACCTCCCTCCTAGCATGA
TGACCCGCAGAAACACCTACGTTTGCACAGAACGCCGGGGGCTGAGCG
CCCGTCACTGTTGCCAAATGGGAAAGAAAACAGCTCAGGCACCCACGG
GTGCCCCCTGCCTCCCCCTCCAGTCACAGCCTGGCACCCCATCAGGGG
AGCGGAGCCGCTGGCACGCGGTTCCACCATCCGCAGCACCTTCCATGG
TGGCCAGGTCCGGGACCGGCGGGCAGGGGGTGGGGGTGGTGGGGGTGTG
CAGAATGGGCCCCCTGCCTCTCCCACACTGGCCCATGAGGCTGCACCCC
TGCCCGCCGGGCGGCCCGCCCCACCACCAACCTCTTACCAAGCTGAC
CTCCAAACTGACCCGAAGGGTCGCAGACGAACCTGAGAGAATCGGGGA
CCTGAGGTCACAAGTTGCCATCTACCTTGGGATCAAACGGAAACCGCCC
CCCGGCTGCTCCGATTCCCCTGGAGTGTGAAGCTGACCAGCTCGCGCCC
TCCTGAGGCCCTGATGGCAGCTCTGCGCCAGGCCACAGCAGCCGCCCGC
TGCCGCTGCCGCCAGCCACAGCCGTTCTGCTGGCCTGCCTGCACGGGG
GTGCGGGCGGGCCCGAGCCCTGTCCCACTTCGAAGTGGAGGTCTGCCA
GCTGCCCCGGCCAGGCTTGCGGGGAGTTCTCTTCCGCCGTGTGGCGGGC
ACCGCCCTGGCCTTCCGCACCTCGTCACCCGCATCTCCAACGACCTC
GAGCTctgaggatcc