Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MARK1 [2 - 795]

Enzyme description:- MARK1 [2 – 795]

Clone number:- DU 1272

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Calculated molecular mass:-

Monoisotopic 92, 529.72 daltons
Average Mass 92, 587.41 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.24

Purity:- 80 %

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

Division of Signal Tranduction Therapy

Clone Data Sheet

MARK1 [2 - 795]

Protein	MARK1 [2 – 795]
Clone Number	DU 1272
Species	Human
Accession number	AF154845
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMDPEF**SARTPLPTVNERDTENHTS VDGYTEPHIQPTKSSSRQNIPRCRNSITSATDEQPHIGNYRLQKTIGKG NFAKVKLARHVLTGREVAVKIIDKTQLNPTSLQKLFREVRIMKILNHPN IVKLFEVIETEKTLYLVMEYASGGEVFDYLVAHGRMKEKEARAKFRQIV SAVQYCHQKYIVHRDLKAENLLLDGDMNIKIADFGFSNEFTVGNKLDTF CGSPPYAAPELFQGKKYDGPEVDVWSLGVILYTLVSGSLPFDGQNLKEL RERVLRGKYRIPFYMSTDCENLLKKLLVLNPIKRGSLEQIMKDRWMNVG HEEEELKPYTEPDPDFNDTKRIDIMVTMGFARDEINDALINQKYDEVMA TYILLGRKPPEFEGGESLSSGNLCQRSRPSSDLNNSTLQSPAHLKVQRS ISANQKQRRFSDHAGPSIPPAVSYTKRPQANSVESEQKEEWDKDVARKL GSTTVGSKSEMTASPLVGPERKKSSTIPSNNVYSGGSMARRNTYVCERT TDRYVALQNGKDSSLTEMSVSSISSAGSSVASAVPSARPRHQKSMSTSG HPIKVTLPTIKDGSEAYRPGTTQRVPAASPSAHSISTATPDRTRFPRGS SSRSTFHGEQLRERRSVAYNGPPASPSHETGAFAHARRGTSTGIISKIT SKFVRRDPSEGEASGRTDTSRSTSGEPKERDKEEGKDSKPRSLRFTWSM KTTSSMDPNDMMREIRKVLDANNCDYEQKERFLLFCVHGDARQDSLVQW EMEVCKLPRLSLNGVRFKRISGTSIAFKNIASKIANELKL**
Native sequence	Amino acids S2 – L795 (end) of human MARK1. Residue S31 of fusion protein is equilivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10. The following amino acid substitutions are present: V – E, where V16 of the native enzyme is E45 of the fusion protein T – S, where T20 of the native enzyme is S49 of the fusion protein
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Eco*R1 site of pFastBAC HTa

Nucleotide sequence of insert

gaattcTCGGCCCGGACGCCATTGCCGACGGTGAACGAGCGGGACACGG
AAAATCATACATCTGTGGATGGATATACTGAACCACACATCCAGCCTAC
CAAGTCGAGTAGCAGACAGAACATCCCCCGGTGTAGAAACTCCATTACG
TCAGCAACAGATGAACAGCCTCACATTGGAAATTACCGTTTACAAAAAA
CAATAGGGAAGGGAAATTTTGCCAAAGTCAAATTGGCAAGACACGTTCT
AACTGGTAGAGAGGTTGCTGTGAAAATAATAGACAAAACTCAGCTAAAT
CCTACCAGTCTACAAAAGTTATTTCGAGAAGTACGAATAATGAAGATAC
TGAATCATCCTAATATAGTAAAATTGTTTGAAGTTATTGAAACAGAGAA
GACTCTCTATTTAGTCATGGAATACGCGAGTGGGGGTGAAGTATTTGAT
TACTTAGTTGCCCATGGAAGAATGAAAGAGAAAGAGGCCCGTGCAAAAT
TTAGGCAGATTGTATCTGCTGTACAGTATTGTCATCAAAAGTACATTGT
TCACCGTGATCTTAAGGCTGAAAACCTTCTCCTTGATGGTGATATGAAT
ATTAAAATTGCTGACTTTGGTTTTAGTAATGAATTTACAGTTGGGAACA
AATTGGACACATTTTGTGGAAGCCCACCCTATGCTGCTCCCGAGCTTTT
CCAAGGAAAGAAGTATGATGGGCCTGAAGTGGATGTGTGGAGTCTGGGC
GTCATTCTCTATACATTAGTCAGTGGCTCCTTGCCTTTCGATGGCCAGA
ATTTAAAGGAACTGCGAGAGCGAGTTTTACGAGGGAAGTACCGTATTCC
CTTCTATATGTCCACAGACTGTGAAAATCTTCTGAAGAAATTATTAGTC
CTGAATCCAATAAAGAGAGGCAGCTTGGAACAAATAATGAAAGATCGAT
GGATGAATGTTGGTCATGAAGAGGAAGAACTAAAGCCATATACTGAGCC
TGATCCGGATTTCAATGACACAAAAAGAATAGACATTATGGTCACCATG
GGCTTTGCACGAGATGAAATAAATGATGCCTTAATAAATCAGAAGTATG
ATGAAGTTATGGCTACTTATATTCTTCTAGGTAGAAAACCACCTGAATT
TGAAGGTGGTGAATCGTTATCCAGTGGAAACTTGTGTCAGAGGTCCCGG
CCCAGTAGTGACTTAAACAACAGCACTCTTCAGTCCCCTGCTCACCTGA
AGGTCCAGAGAAGTATCTCAGCAAATCAGAAGCAGCGGCGTTTCAGTGA
TCATGCTGGTCCATCCATTCCTCCTGCTGTATCATATACCAAAAGACCT
CAGGCTAACAGTGTGGAAAGTGAACAGAAAGAGGAGTGGGACAAAGATG
TGGCTCGAAAACTTGGCAGCACAACAGTTGGATCAAAAAGCGAGATGAC
TGCAAGCCCTCTTGTAGGGCCAGAGAGGAAAAAATCTTCAACTATTCCA
AGTAACAATGTGTATTCTGGAGGTAGCATGGCAAGAAGGAATACATATG
TCTGTGAAAGGACCACAGATCGATACGTAGCATTGCAGAATGGAAAAGA
CAGCAGCCTTACGGAGATGTCTGTGAGTAGCATATCTTCTGCAGGCTCT
TCTGTGGCCTCTGCTGTCCCCTCAGCACGACCCCGCCACCAGAAGTCCA
TGTCCACTTCTGGTCATCCTATTAAAGTCACACTGCCAACCATTAAAGA
CGGCTCTGAAGCTTACCGGCCTGGTACAACCCAGAGAGTGCCTGCTGCT
TCCCCATCTGCTCACAGTATTAGTACTGCGACTCCAGACCGGACCCGTT
TTCCCCGAGGGAGCTCAAGCCGAAGCACTTTCCATGGTGAACAGCTCCG
GGAGCGACGCAGCGTTGCTTATAATGGGCCACCTGCTTCACCATCCCAT
GAAACGGGTGCATTTGCACATGCCAGAAGGGGAACGTCAACTGGTATAA
TAAGCAAAATCACATCCAAATTTGTTCGCAGGGATCCAAGTGAAGGCGA
AGCCAGTGGCAGAACCGACACCTCAAGAAGTACATCAGGGGAACCAAAA
GAAAGAGACAAGGAAGAGGGTAAAGATTCTAAGCCGCGTTCTTTGCGGT
TCACATGGAGTATGAAGACCACTAGTTCAATGGACCCTAATGACATGAT
GAGAGAAATCCGAAAAGTGTTAGATGCAAATAACTGTGATTATGAGCAA
AAAGAGAGATTTTTGCTTTTCTGTGTCCATGGAGACGCTAGACAGGATA
GCCTCGTGCAGTGGGAGATGGAAGTCTGCAAGTTGCCACGACTGTCACT

TAATGGGGTTCGCTTCAAGCGAATATCTGGGACATCTATTGCCTTTAAG
AACATTGCATCAAAAATAGCAAATGAGCTTAAGCTGtaagaattc