

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MARK1 [2 - 795]

<u>Enzyme description:-</u>	MARK1 [2 – 795]
<u>Clone number:-</u>	DU 1272
<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>	92, 529 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

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Clone Data Sheet

MARK1 [2 - 795]

<u>Protein</u>	MARK1 [2 – 795]
<u>Clone Number</u>	DU 1272
<u>Species</u>	Human
<u>Accession number</u>	AF154845
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMDPEFSARTPLPTVNERTENHTS VDGYTEPHIQPTKSSSRQNIPRCRNSITSATDEQPHIGNYRLQKTIGKG NFAKVKLARHVLTGREVAVKIIDKTQLNPTSLQKLFREVRIMKILNHPN IVKLFEVIETEKTLYLVMEYASGGEVFDYLVAHGRMKEKEARAKFRQIV SAVQYCHQKYIVHRDLKAENLLLDGDMNIKIADFGFSNEFTVGNKLDTF CGSPPYAAPELFQGGKYDGPEVDVWSLGVILYTLVSGSLPFDGQNLKEL RERVLRGKYRIPFYMSTDCENLLKLLVLNPIKRGSLEQIMKDRWMNVG HEEEELKPYTEPDPDFNDTKRIDIMVTMGFARDEINDALINQKYDEVMA TYILLGRKPPEFEGGESLSSGNLCQRSRPSSDLNNSTLQSPAHLKVQRS ISANQKQRRFSDHAGPSIPPAVSYTKRPQANSVESEQKEEWDKDVARKL GSTTVGSKSEMTASPLVGPERKKSSTIPSNNVYSGGSMARRNTYVCERT TDRYVALQNGKDSSLTEMSVSSISSAGSSVASAVPSARPRHQKSMSTSG HPIKVTLPTIKDGSEAYRPGTTQRVPAASPSAHSISTATPDRTRFPRGS SSRSTFHGEQLRERRSVAYNGPPASPSHETGAFAHARRGTSTGIISKIT SKFVRRDPSEGEASGRDTSRSTSGEPKERDKEEGKDSKPRSLRFTWSM KTTSSMDPNDMMREIRKVLDANNCDYEQKERFLLFCVHGDARQDSLVQW EMEVCKLPRLSLNGVRFKRISGTSIAFKNIASKIANELKL
<u>Native sequence</u>	Amino acids S2 – L795 (end) of human MARK1. Residue S31 of fusion protein is equivalent 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
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	<i>Eco</i> R1 site of pFastBAC HTa

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Nucleotide sequence of insert

gaattcTCGGCCCGGACGCCATTGCCGACGGTGAACGAGCGGGACACGG
AAAATCATACATCTGTGGATGGATATACTGAACCACACATCCAGCCTAC
CAAGTCGAGTAGCAGACAGAACATCCCCCGGTGTAGAACTCCATTACG
TCAGCAACAGATGAACAGCCTCACATTGGAAATTACCGTTTACAAAAA
CAATAGGGAAGGGAAATTTTGCCAAAGTCAAATTGGCAAGACACGTTCT
AACTGGTAGAGAGGTTGCTGTGAAAATAATAGACAAAACCTCAGCTAAAT
CCTACCAGTCTACAAAAGTTATTTTCGAGAAGTACGAATAATGAAGATAC
TGAATCATCCTAATATAGTAAAATTGTTTGAAGTTATTGAAACAGAGAA
GACTCTCTATTTAGTCATGGAATACGCGAGTGGGGGTGAAGTATTTGAT
TACTTAGTTGCCCATGGAAGAATGAAAGAGAAAGAGGGCCCGTGCAAAT
TTAGGCAGATTGTATCTGCTGTACAGTATTGTCATCAAAGTACATTGT
TCACCGTGATCTTAAGGCTGAAAACCTTCTCCTTGATGGTGATATGAAT
ATTAAAATTGCTGACTTTGGTTTTAGTAATGAATTTACAGTTGGGAACA
AATTGGACACATTTTGTGGAAGCCACCCTATGCTGCTCCCGAGCTTTT
CCAAGGAAAGAAGTATGATGGGCCTGAAGTGGATGTGTGGAGTCTGGGC
GTCATTCTCTATACATTAGTCAGTGGCTCCTTGCCCTTTCGATGGCCAGA
ATTTAAAGGAAGTGCAGAGCGAGTTTTACGAGGGAAGTACCGTATTCC
CTTCTATATGTCCACAGACTGTGAAAATCTTCTGAAGAAATTATTAGTC
CTGAATCCAATAAAGAGAGGCAGCTTGGAACAAATAATGAAAGATCGAT
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TGTCCACTTCTGGTCATCCTATTAAAGTCACACTGCCAACCATTAAAGA
CGGCTCTGAAGCTTACCGGCCTGGTACAACCCAGAGAGTGCCTGCTGCT
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TTCCCCGAGGGAGCTCAAGCCGAAGCACTTTCCATGGTGAACAGCTCCG
GGAGCGACGCAGCGTTGCTTATAATGGGCCACCTGCTTCACCATCCCAT
GAAACGGGTGCATTTGCACATGCCAGAAGGGGAACGTCAACTGGTATAA
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AGCCAGTGGCAGAACCGACACCTCAAGAAGTACATCAGGGGAACCAAAA
GAAAGAGACAAGGAAGAGGGTAAAGATTCTAAGCCGCGTTCTTTGCGGT
TCACATGGAGTATGAAGACCACTAGTTCAATGGACCCTAATGACATGAT
GAGAGAAATCCGAAAAGTGTTAGATGCAAATAACTGTGATTATGAGCAA
AAAGAGAGATTTTTGCTTTTCTGTGTCCATGGAGACGCTAGACAGGATA
GCCTCGTGAGTGGGAGATGGAAGTCTGCAAGTTGCCACGACTGTCACT

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TAATGGGGTTCGCTTCAAGCGAATATCTGGGACATCTATTGCCTTTAAG
AACATTGCATCAAAAATAGCAAATGAGCTTAAGCTGtaagaattc