

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MARK2 [2 - 691]

<u>Enzyme description:-</u>	MARK2 [2 – 691]
<u>Clone number:-</u>	DU 1269
<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

Calculated molecular mass:-

Monoisotopic 81, 780.68 daltons
Average Mass 81, 831.82 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.51

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 [KKKVSRSGLYRSPSPENLNRPR]
Final concentration: 300 µM

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Clone Data Sheet

MARK2 [2 - 691]

<u>Protein</u>	MARK2 [2 – 691]
<u>Clone Number</u>	DU 1269
<u>Species</u>	Human
<u>Accession number</u>	NM_004954
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGIRNSKAYVDIRGRNSATSAD EQPHIGNYRLLKTIGKGNFAKVKLARHILTGKEVAVKIIDKTQLNSS SLQKLFREVRIMKVLNHPNIVKLFVETETKTLVMEYASGGEVFDY LVAHRMKEKEARAKFRQIVSAVQYCHQKFIVHRDLKAENLLLDAD MNIKIADF GFSNEFTFGNKLDTF CGSPPYAAPELFQGGKYDGPE VDVWSLGVILYTLVSGSLPFDGQNLKELRERVLRGKYRIPFYMSTDC ENLLKKFLILNPSKR GTLEQIMKDRWMNVGHEDDELKPYVEPLPD YKDPRTTELMVSMGYTREE IQDSL VGQRYNEVMATYLLGYKSSE LEGDTITLKPRPSADLTNSSAPS PSHKVQRSVSANPKQRRFSDQAG PAIPTSNSYSKKTQSNNAENKRPEED RESGRKASSTAKVPASPLP GLERKKTTPSTNSVLSTSTNRSRNSPLL ERASLGQASIQNGKDSTAP QRPVPASPSAHNISSSGGAPDRTNFPRGVS SRSTFHAGQLRQVRDQ QNLPGVTPASPSGHSQGRRGASGSIFS KFTSK FVRRNLNEPESKDR VETLRPHVVGSGGNDKEKEEFREAKPRSLRFTWSM KTTSSMEPNEM MREIRKVL DANCQSELHEKYMLLCMHGTPGHEDFVQW EMEVCKLPRL SLNGVRFKRISGTSM AFKNIASKIANELKL
<u>Native sequence</u>	Amino acids I2 – L691 (end) of human MARK2. Residue I37 of fusion protein is equivalent to I2 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>	<i>Sal</i> I site of pFastBAC HTc

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Nucleotide sequence of insert

gtcgcacATTCTGGGGCCGCAACTCAGCCACCTCTGCTGATGAGCAGCCCC
ACATTGGAAACTACCGGCTCCTCAAGACCATTGGCAAGGGTAATTTTGC
CAAGGTGAAGTTGGCCCCGACACATCCTGACTGGGAAAGAGGTAGCTGTG
AAGATCATTGACAAGACTCAACTGAACTCCTCCAGCCTCCAGAACTAT
TCCGCGAAGTAAGAATAATGAAGGTTTTGAATCATCCCAACATAGTTAA
ATTATTTGAAGTGATTGAGACTGAGAAAACGCTCTACCTTGTCATGGAG
TACGCTAGTGGCGGAGAGGTATTTGATTACCTAGTGGCTCATGGCAGGA
TGAAAGAAAAAGAGGCTCGAGCCAAATTCCGCCAGATAGTGTCTGCTGT
GCAGTACTGTCAACCAGAAGTTTATTGTCCATAGAGACTTAAAGGCAGAA
AACCTGCTCTTGGATGCTGATATGAACATCAAGATTGCAGACTTTGGCT
TCAGCAATGAATTCACCTTTGGGAACAAGCTGGACACCTTCTGTGGCAG
TCCCCCTTATGCTGCCCCAGAACTCTTCCAGGGCAAAAAATATGATGGA
CCCGAGGTGGATGTGTGGAGCCTAGGAGTTATCCTCTATACACTGGTCA
GCGGATCCCTGCCTTTTGTATGGACAGAACCTCAAGGAGCTGCGGGAACG
GGTACTGAGGGGGAAATACCGTATTCCATTCTACATGTCCACGGACTGT
GAAAACCTGCTTAAGAAATTTCTCATTCTTAATCCCAGCAAGAGAGGCA
CTTTAGAGCAAATCATGAAAGATCGATGGATGAATGTGGGTACAGAGA
TGATGAACTAAAGCCTTACGTGGAGCCACTCCCTGACTACAAGGACCCC
CGGCGGACAGAGCTGATGGTGTCCATGGGTTATACACGGGAAGAGATCC
AGGACTCGCTGGTGGGCCAGAGATACAACGAGGTGATGGCCACCTATCT
GCTCCTGGGCTACAAGAGCTCCGAGCTGGAAGGCGACACCATCACCTG
AAACCCCGGCCTTCAGCTGATCTGACCAATAGCAGCGCCCCATCCCCAT
CCCACAAGGTACAGCGCAGCGTGTCTGGCCAATCCCAAGCAGCGGCGCTT
CAGCGACCAGGCTGGTCTTGCCATTCCACCTCTAATTCTTACTCTAAG
AAGACTCAGAGTAACAACGCAGAAAATAAGCGGCCTGAGGAGGACCGGG
AGTCAGGGCGGAAAGCCAGCAGCACAGCCAAGGTGCCTGCCAGCCCCCT
GCCCCGGTCTGGAGAGGAAGAAGACCACCCCAACCCCTCCACGAACAGC
GTCCTCTCCACCAGCACAAATCGAAGCAGGAATTCCCCACTTTTGGAGC
GGGCCAGCCTCGGCCAGGCCTCCATCCAGAATGGCAAAGACAGCACAGC
CCCCCAGCGTGTCCCTGTTGCCTCCCCATCCGCCACAACATCAGCAGC
AGTGGTGGAGCCCCAGACCGAACTAACTTCCCCCGGGGTGTGTCCAGCC
GAAGCACCTTCCATGCTGGGCAGCTCCGACAGGTGCGGGACCAGCAGAA
TTTGCCCTACGGTGTGACCCAGCCTCTCCCTCTGGCCACAGCCAGGGC
CGGCGGGGGCCTCTGGGAGCATCTTCAGCAAGTTCACCTCCAAGTTTG
TACGCAGGAACCTGAATGAACCTGAAAGCAAAGACCGAGTGGAGACGCT
CAGACCTCACGTGGTGGGCAGTGGCGGCAACGACAAAGAAAAGGAAGAA
TTTCGGGAGGCCAAGCCCCGCTCCCTCCGCTTCACGTGGAGTATGAAGA
CCACGAGCTCCATGGAGCCCAACGAGATGATGCGGGAGATCCGCAAGGT
GCTGGACGCGAACAGCTGCCAGAGCGAGCTGCATGAGAAGTACATGCTG
CTGTGCATGCACGGCACGCCGGGCCACGAGGACTTCGTGCAGTGGGAGA
TGGAGGTGTGCAAACCTGCCGCGGCTCTCTCTCAACGGGGTTTCGATTTAA
GCGGATATCGGGCACCTCCATGGCCTTCAAAAACATTGCCTCCAAAATA
GCCAACGAGCTGAAGCTTtaagtcgcac