

Standard Operating Procedure

Preparation of active MARK3 [2 - 729]

<u>Enzyme description:-</u>	MARK3 [2 - 729]
<u>Clone number:-</u>	DU 1296
<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>	86,036 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

CLONE DATA SHEET - MARK3 [2 - 729]

Protein MARK3 [2 – 729]

Clone Number DU 1296

Species Human

Accession number U64205

Tags N-terminal His(6)

Baculovirus expressed protein

MSYYHHHHHHHDYDIPTTENLYFQGAMGSGIQRPTSTSSLVAAASTRTPL
PTVNERDTENHTSHGDGRQEVTSRTSRSGARCRNSIASCADQPHIGNY
RLLKTIGKGNFAKVKLARHILTGREVAIKIIDKTQLNPTSLQKLFREVR
IMKILNHPNIVKLFVETIEKTLYLIMEYASGGEVFDYLVAHGRMKEKE
ARSKFRQIVSAVQYCHQKRIVHRDLKAENLLLDADMNIKIADFGFSNEF
TVGGKLDTCGSPPYAAPELFQGGKYDGPEVDVWSLGVILYTLVSGSLP
FDGQNLKELRERVLRGKYRIPFYMSTDCENLLKRFLVLNPIKRGTLQI
MKDRWINAGHEEDELKPFVEPELDISDQKRIDIMVGMGYSQEEIQESLS
KMKYDEITATYLLLGRKSSSELDASDSSSSSNLSLAKVRPSSDLNNSTGQ
SPHHKVQRSVSSSQKQRRYSDHAGPAIPSVVAYPKRSQTSTADGDLKED
GISSRKSSGSAVGGKGIAPASPM LGNASNPKNADI PERKKSSTVPSSNT
ASGGMTRRNTYVCSERTTADRHSVIQNGKENSTIPDQRTPVASTHSISS
AATPDRI RFPRGTASRSTFHGQPRERRTATYNGPPASPSLSHEATPLSQ
TRSRGSTNLF SKLTSKLTRSRNVSAEQKDENEAKPRSLRFTWSMKTTTS
SMDPGDMMREIRKVL DANNC DYEQRERFLLFCVHGDGHAENLVQWEMEV
CKLPRLSLNGVRFRKISGTSIAFKNIASKIANELKL

Native sequence Amino acids S2 – L729 (end) of human MARK3.
Residue S44 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 *Not1* site of pFastBAC HTb

**Nucleotide
sequence of
insert**

gcggccgcctCCACTAGGACCCCATTTGCCAACGGTGAATGAACGAGACA
CTGAAAACACACGTACATGGAGATGGGCGTCAAGAAGTTACCTCTCG
TACCAGCCGCTCAGGAGCTCGGTGTAGAACTCTATAGCCTCCTGTGCA
GATGAACAACCTCACATCGGAACTACAGACTGTTGAAAACAATCGGCA
AGGGGAATTTTGCAAAAGTAAAAATTGGCAAGACATATCCTTACAGGCAG
AGAGGTTGCAATAAAAAATAATTGACAAAACCTCAGTTGAATCCAACAAGT
CTACAAAAGCTCTTCAGAGAAGTAAGAATAATGAAGATTTTAAATCATC
CCAATATAGTGAAGTTATTCGAAGTCATTGAACTGAAAAACACTCTA
CCTAATCATGGAATATGCAAGTGGAGGTGAAGTATTTGACTATTTGGTT
GCACATGGCAGGATGAAGGAAAAAGAAGCAAGATCTAAATTTAGACAGA
TTGTGTCTGCAGTTCAATACTGCCATCAGAAACGGATCGTACATCGAGA
CCTCAAGGCTGAAAATCTATTGTTAGATGCCGATATGAACATTTAAATA
GCAGATTTTCGGTTTTAGCAATGAATTTACTGTTGGCGGTAAACTCGACA
CGTTTTGTGGCAGTCCTCCATACGCAGCACCTGAGCTCTTCCAGGGCAA
GAAATATGACGGGCCAGAAGTGGATGTGTGGAGTCTGGGGGTCAATTTA
TACACACTAGTCAGTGGCTCACTTCCCTTTGATGGGCAAAACCTAAAGG
AACTGAGAGAGAGAGTATTAAGAGGGAAATACAGAATTCCTTCTACAT
GTCTACAGACTGTGAAAACCTTCTCAAACGTTTCTTGGTGCTAAATCCA
ATTAAACGCGCACTCTAGAGCAAATCATGAAGGACAGGTGGATCAATG
CAGGGCATGAAGAAGATGAACTCAAACCATTTGTTGAACCAGAGCTAGA
CATCTCAGACCAAAAAAGAATAGATATTATGGTGGGAATGGGATATTCA
CAAGAAGAAATTCAAGAATCTCTTAGTAAGATGAAATACGATGAAATCA
CAGCTACATATTTGTTATTGGGGAGAAAATCTTCAGAGCTGGATGCTAG
TGATTCCAGTTCTAGCAGCAATCTTTCACTTGCTAAGGTTAGGCCGAGC
AGTGATCTCAACAACAGTACTGGCCAGTCTCCTCACCACAAAGTGCAGA
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ACCAGCTATTCCTTCTGTTGTGGCGTATCCGAAAAGGAGTCAGACAAGC
ACTGCAGATGGTGACCTCAAAGAAGATGGAATTTCTTCCCGGAAATCAA
GTGGCAGTGCTGTTGGAGGAAAGGGAATTGCTCCAGCCAGTCCCATGCT
TGGGAATGCAAGTAATCCTAATAAGGCGGATATTCTTGAACGCAAGAAA
AGCTCCACTGTCCCTAGTAGTAACACAGCATCTGGTGGAATGACACGAC
GAAATACTTATGTTTGCAGTGAGAGAACTACAGCTGATAGACACTCAGT
GATTCAGAATGGCAAAGAAAACAGCACTATTCTTGATCAGAGAACTCCA
GTTGCTTCAACACACAGTATCAGTAGTGCAGCCACCCAGATCGAATCC
GCTTCCCAAGAGGCACTGCCAGTCGTAGCACTTTCCACGGCCAGCCCCG
GGAACGGCGAACCGCAACATATAATGGCCCTCCTGCCTCTCCAGCCTG
TCCCATGAAGCCACACCATTTGTCCCAGACTCGAAGCCGAGGCTCCACTA
ATCTCTTTAGTAAATTAACCTCAAACCTCACAAGGAGTCGCAATGTATC
TGCTGAGCAAAAAGATGAAAACAAAGAAGCAAAGCCTCGATCCCTACGC
TTCACCTGGAGCATGAAAACCACTAGTTCAATGGATCCCGGGGACATGA
TGCGGGAAATCCGCAAAGTGTTGGACGCCAATAACTGCGACTATGAGCA
GAGGGAGCGCTTCTTGCTCTTCTGCGTCCACGGAGATGGGCACGCGGAG
AACCTCGTGCAGTGGGAAATGGAAGTGTGCAAGCTGCCAAGACTGTCT
CTGAACGGGGTCCGGTTTAAGCGGATATCGGGGACATCCATAGCCTTCA
AAAATATTGCTTCCAAAATTGCCAATGAGCTAAAGCTGtaagcggccgc