

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MST3 isoform A [1 – 431]

Enzyme description:- MST3 isoform A [1 - 431]

Clone number:- DU 62878

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Cobalt Agarose

Calculated molecular mass:-

Monoisotopic 51, 774.31 daltons

Average Mass 51, 806.61 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.42

Purity:- 85 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

Myelin Basic Protein (MBP)

Final concentration: 0.33 mg/ml

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Clone Data Sheet

MST3 isoform A [1 - 431]

<u>Protein</u>	MST3 isoform A [1 - 431]
<u>Clone number</u>	DU 62878
<u>Species</u>	Human
<u>Accession number</u>	Q9Y6E0-2
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	MGSSHHHHHHSSGLEVLFGPGSKLEFASLETSEAAAMAHSPVQSGLPG MQNLKADPEELFTKLEKIGKGSFGEVFKGIDNRTQKVVAIKIIDLEEAE DEIEDIQQEITVLSQCDSFYVTKYYGSYLKDTKLWIIMEYLGGSALDL LEPGPLDETQIATILREILKGLDYLHSEKKIHRDIKAANVLLSEHGEVK LADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGIT AIELARGEPPHSELHPMKVFLIPKNNPPTLEGNYSKPLKEFVEACLNK EPSFRPTAKELLKHKFILRNAKTSYLTTELIDRYKRWKAEQSHDDSSSE DSDAETDGQASGSDSGDWIFTIREKDPKNLENGALQPSDLDRNKMKDI PKRPFSQCLSTIISPLFAELKEKSQACGGNLSIEELRGAIYLAEEACP GISDTMVAQLVQRLQRYSLSGGGTSSH
<u>Native sequence</u>	Amino acids M1 – H431 (end) of human MST3 isoform A. Residue M38 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 5 – 10.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 14 - 21

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Nucleotide

Sequence of insert

gcggccgcgATGGCTCACTCCCCGGTGCAGTCGGGCCTGCCCCGGCATGC
AGAACCTAAAAGCAGACCCAGAAGAGCTTTTTACAAAAGCTAGAGAAAAT
TGGGAAGGGCTCCTTTGGAGAGGTGTTCAAAGGCATTGACAATCGGACT
CAGAAAGTGGTTGCCATAAAGATCATTGATCTGGAAGAAGCTGAAGATG
AGATAGAGGACATTCAACAAGAAATCACAGTGCTGAGTCAGTGTGACAG
TCCATATGTAACCAAATATTATGGATCCTATCTGAAGGATACAAAATTA
TGGATAATAATGGAATATCTTGGTGGAGGCTCCGCACTAGATCTATTAG
AACCTGGCCCATTAGATGAAACCCAGATCGCTACTATATTAAGAGAAAT
ACTGAAAGGACTCGATTATCTCCATTCCGAGAAAGAAAATCCACAGAGAC
ATTAAAGCGGCCAACGTCCTGCTGTCTGAGCATGGCGAGGTGAAGCTGG
CGGACTTTGGCGTGGCTGGCCAGCTGACAGACACCCAGATCAAAAGGAA
CACCTTCGTGGGCACCCCATCTCTGGATGGCACCCGAGGTCATCAAACAG
TCGGCCTATGACTCGAAGGCAGACATCTGGTCCCTGGGCATAACAGCTA
TTGAACTTGCAAGAGGGGAACACCTCATTCCGAGCTGCACCCCATGAA
AGTTTTATTCTCATTCCAAAGAACAACCCACCGACGTTGGAAGGAAAC
TACAGTAAACCCCTCAAGGAGTTTGTGGAGGCCTGTTTGAATAAGGAGC
CGAGCTTTAGACCCACTGCTAAGGAGTTATTGAAGCACAAGTTTATACT
ACGCAATGCAAAGAAAACCTCCTACTTGACCGAGCTCATCGACAGGTAC
AAGAGATGGAAGGCCGAGCAGAGCCATGACGACTCGAGCTCCGAGGATT
CCGACGCGGAAACAGATGGCCAAGCCTCGGGGGGCAGTGATTCTGGGGA
CTGGATCTTCACAATCCGAGAAAAAGATCCCAAGAATCTCGAGAATGGA
GCTCTTCAGCCATCGGACTTGGACAGAAATAAGATGAAAGACATCCCAA
AGAGGCCTTTCTCTCAGTGTTTATCTACAATTATTTCTCCTCTGTTTGC
AGAGTTGAAGGAGAAGAGCCAGGCGTGCGGAGGGAAGTTGGGGTCCATT
GAAGAGCTGCGAGGGGCCATCTACCTAGCGGAGGAGGCGTGCCCTGGCA
TCTCCGACACCATGGTGGCCAGCTCGTGCAGCGGCTCCAGAGATACTC
TCTAAGTGGTGGAGGAACTTCATCCCACtgagcggccgc