

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MST3 [1 - 431]

Enzyme description:- MST3 [1 - 431]

Clone number:- DU 30889

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- Glutathione Sepharose

Calculated molecular mass:-

Monoisotopic 75,696.68 daltons

Average Mass 75,744.71 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pl:- 5.47

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1mM 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 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

Myelin Basic Protein (MBP)

Final concentration: 0.3 mg/ml

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Clone Data Sheet

MST3 [1 - 431]

<u>Protein</u>	MST3 [1 - 431]
<u>Clone number</u>	DU 30889
<u>Species</u>	Human
<u>Accession number</u>	AAH65378
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPGIPGSTRAAAMAH SPVQSGLPGMQNLKADPEELFTKLEKIGKGSFGEVFKGIDNRTQKVVAI KIIDLEEADEIEDIQQEITVLSQCDSFYVTKYYGSYLKDTKLWIMEY LGGGSALDLLEPGPLDETQIATILREILKGLDYHSEKKIHRDIKAANV LLSEHGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQ SAYDSK ADIWSLGITAIELARGEPPHSELHPMKVFLIPKNNPPTLEGNYSKPLK EFVEACLNKEPSFRPTAKELLKHKFILRNAKKT SYLTEIDRYKRWKA E QSHDDSSSESDAETDGQASGGSDSGDWIFTIREKDPKNLENGALQPSD LDRNKMKDIPKRPF SQCLSTIISPLFAELKEKSQACGGNLGSIEELRGV IYLAEEACPGISDTMVAQLVQRLQRYSLSGGGTSSH
<u>Native sequence</u>	Amino acids M1 – H431 (end) of human MST3. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Not</i> I and <i>Not</i> I sites in pFastBAC GST

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Nucleotide sequence of insert

gcggccgcgATGGCTCACTCCCCGGTGCAGTCGGGCCTGCCCGGCATGC
AGAACCTAAAAGCAGACCCAGAAGAGCTTTTTTACAAAACCTAGAGAAAAT
TGGGAAGGGCTCCTTTGGAGAGGTGTTCAAAGGCATTGACAATCGGACT
CAGAAAGTGGTTGCCATAAAGATCATTGATCTGGAAGAAGCTGAAGATG
AGATAGAGGACATTCAACAAGAAATCACAGTGCTGAGTCAGTGTGACAG
TCCATATGTAACCAAATATTATGGATCCTATCTGAAGGATACAAAATTA
TGGATAATAATGGAATATCTTGGTGGAGGCTCCGCACTAGATCTATTAG
AACCTGGCCCATTAGATGAAACCCAGATCGCTACTATATTAAGAGAAAT
ACTGAAAGGACTCGATTATCTCCATTTCGGAGAAGAAAATCCACAGAGAC
ATTAAAGCGGCCAACGTCCTGCTGTCTGAGCATGGCGAGGTGAAGCTGG
CGGACTTTGGCGTGGCTGGCCAGCTGACAGACACCCAGATCAAAAGGAA
CACCTTCGTGGGCACCCCATCTGGATGGCACCCGAGGTCATCAAACAG
TCGGCCTATGACTCGAAGGCAGACATCTGGTCCCTGGGCATAACAGCTA
TTGAACTTGCAAGAGGGGAACCACTCATTCCGAGCTGCACCCCATGAA
AGTTTTATTTCCTCATTCCAAAGAACAACCCACCGACGTTGGAAGGAAAC
TACAGTAAACCCCTCAAGGAGTTTGTGGAGGCCTGTTTGAATAAGGAGC
CGAGCTTTAGACCCACTGCTAAGGAGTTATTGAAGCACAAAGTTTATACT
ACGCAATGCAAAGAAAACCTCCTACTTGACCGAGCTCATCGACAGGTAC
AAGAGATGGAAGGCCGAGCAGAGCCATGACGACTCGAGCTCCGAGGATT
CCGACGCGGAAACAGATGGCCAAGCCTCGGGGGGCAGTGATTCTGGGGA
CTGGATCTTCACAATCCGAGAAAAAGATCCCAAGAATCTCGAGAATGGA
GCTCTTCAGCCATCGGACTTGGACAGAAATAAGATGAAAGACATCCCAA
AGAGGCCTTTCTCTCAGTGTTTATCTACAATTATTTCTCCTCTGTTTGC
AGAGTTGAAGGAGAAGAGCCAGGCGTGCGGAGGGAAGTTGGGGTCCATT
GAAGAGCTGCGAGGGGTCATCTACCTAGCGGAGGAGGCGTGCCCTGGCA
TCTCCGACACCATGGTGGCCAGCTCGTGCAGCGGCTCCAGAGATACTC
TCTAAGTGGTGGAGGAACCTTCATCCCACtgcggccgc