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 pI:- 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]

Protein	MST3 [1 - 431]
Clone number	DU 30889
Species	Human
Accession number	AAH65378
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA**MAH** **SPVQSGLPGMQNLKADPEELFTKLEKIGKGSFGEVFKGIDNRTQKVVAI** **KIIDLEEAEDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTKLWIIMEY** **LGGGSALDLLEPGPLDETQIATILREILKGLDYLHSEKKIHRDIKAANV** **LLSEHGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSK** **ADIWSLGITAIELARGEPPHSELHPMKVLFLIPKNNPPTLEGNYSKPLK** **EFVEACLNKEPSFRPTAKELLKHKFILRNAKKTSYLTELIDRYKRWKAE** **QSHDDSSSEDSDAETDGQASGGSDSGDWIFTIREKDPKNLENGALQPSD** **LDRNKMKDIPKRPFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRGV** **IYLAEEACPGISDTMVAQLVQRLQRYSLSGGGTSSH**
Native sequence	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.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Not*1 and *Not*1 sites in pFastBAC GST

Nucleotide sequence of insert

gcggccgcgATGGCTCACTCCCCGGTGCAGTCGGGCCTGCCCGGCATGC
AGAACCTAAAAGCAGACCCAGAAGAGCTTTTTACAAAACTAGAGAAAAT
TGGGAAGGGCTCCTTTGGAGAGGTGTTCAAAGGCATTGACAATCGGACT
CAGAAAGTGGTTGCCATAAAGATCATTGATCTGGAAGAAGCTGAAGATG
AGATAGAGGACATTCAACAAGAAATCACAGTGCTGAGTCAGTGTGACAG
TCCATATGTAACCAAATATTATGGATCCTATCTGAAGGATACAAAATTA
TGGATAATAATGGAATATCTTGGTGGAGGCTCCGCACTAGATCTATTAG
AACCTGGCCCATTAGATGAAACCCAGATCGCTACTATATTAAGAGAAAT
ACTGAAAGGACTCGATTATCTCCATTCGGAGAAGAAAATCCACAGAGAC
ATTAAAGCGGCCAACGTCCTGCTGTCTGAGCATGGCGAGGTGAAGCTGG
CGGACTTTGGCGTGGCTGGCCAGCTGACAGACACCCAGATCAAAAGGAA
CACCTTCGTGGGCACCCCATTCTGGATGGCACCCGAGGTCATCAAACAG
TCGGCCTATGACTCGAAGGCAGACATCTGGTCCCTGGGCATAACAGCTA
TTGAACTTGCAAGAGGGGAACCACCTCATTCCGAGCTGCACCCCATGAA
AGTTTTATTCCTCATTCCAAAGAACAACCCACCGACGTTGGAAGGAAAC
TACAGTAAACCCCTCAAGGAGTTTGTGGAGGCCTGTTTGAATAAGGAGC
CGAGCTTTAGACCCACTGCTAAGGAGTTATTGAAGCACAAGTTTATACT
ACGCAATGCAAAGAAAACTTCCTACTTGACCGAGCTCATCGACAGGTAC
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CCGACGCGGAAACAGATGGCCAAGCCTCGGGGGGCAGTGATTCTGGGGA
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GCTCTTCAGCCATCGGACTTGGACAGAAATAAGATGAAAGACATCCCAA
AGAGGCCTTTCTCTCAGTGTTTATCTACAATTATTTCTCCTCTGTTTGC
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TCTCCGACACCATGGTGGCCCAGCTCGTGCAGCGGCTCCAGAGATACTC
TCTAAGTGGTGGAGGAACTTCATCCCACtgagcggccgc