Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MST1 [1 - 487]

Enzyme description:-	MST1 [1 - 487]
Clone number:-	DU 30863
Source:-	Recombinant
Expression system:-	Baculovirus expression vector system
Tag:-	N-terminal GST
Purification method:-	Glutathione Sepharose

Calculated molecular mass:-

Monoisotopic	82, 401.50 daltons
Average Mass	82, 454.32 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	5.15
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

MST1 [1 - 487]

Protein	MST1 [1 - 487]
Clone number	DU 30863
Species	Human
Accession number	NM_006282.2
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**METVQLRNPPRRQL** **KKLDEDSLTKQPEEVFDVLEKLGEGSYGSVYKAIHKETGQIVAIKQVPV** **ESDLQEIIKEISIMQQCDSPHVVKYYGSYFKNTDLWIVMEYCGAGSVSD** **IIRLRNKTLTEDEIATILQSTLKGLEYLHFMRKIHRDIKAGNILLNTEG** **HAKLADFGVAGQLTDTMAKRNTVIGTPFWMAPEVIQEIGYNCVADIWSL** **GITAIEMAEGKPPYADIHPMRAIFMIPTNPPPTFRKPELWSDNFTDFVK** **QCLVKSPEQRATATQLLQHPFVRSAKGVSILRDLINEAMDVKLKRQESQ** **QREVDQDDEENSEEDEMDSGTMVRAVGDEMGTVRVASTMTDGANTMIEH** **DDTLPSQLGTMVINAEDEEEEGTMKRRDETMQPAKPSFLEYFEQKEKEN** **QINSFGKSVPGPLKNSSDWKIPQDGDYEFLKSWTVEDLQKRLLALDPMM** **EQEIEEIRQKYQSKRQPILDAIEAKKRRQQNF**
Native sequence	Amino acids M1 – F487 (end) of human MST1. Residue M232 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	*Bam*H1 and *Not*1 sites in pFastBAC GST

Nucleotide sequence of insert

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ggatccATGGAGACGGTACAGCTGAGGAACCCGCCGCGCCGGCAGCTGA
AAAAGTTGGATGAAGATAGTTTAACCAAACAACCAGAAGAAGTATTTGA
TGTCTTAGAGAAACTTGGAGAAGGGTCCTATGGCAGCGTATACAAAGCT
ATTCATAAAGAGACCGGCCAGATTGTTGCTATTAAGCAAGTTCCTGTGG
AATCAGACCTCCAGGAGATAATCAAAGAAATCTCTATAATGCAGCAATG
TGACAGCCCTCATGTAGTCAAATATTATGGCAGTTATTTTAAGAACACA
GACTTATGGATCGTTATGGAGTACTGTGGGGCTGGTTCTGTATCTGATA
TCATTCGATTACGAAATAAAACGTTAACAGAAGATGAAATAGCTACAAT
ATTACAATCAACTCTTAAGGGACTTGAATACCTTCATTTTATGAGAAAA
ATACACCGAGATATCAAGGCAGGAAATATTTTGCTAAATACAGAAGGAC
ATGCAAAACTTGCAGATTTTGGGGTAGCAGGTCAACTTACAGATACCAT
GGCCAAGCGGAATACAGTGATAGGAACACCATTTTGGATGGCTCCAGAA
GTGATTCAGGAAATTGGATACAACTGTGTAGCAGACATCTGGTCCCTGG
GAATAACTGCCATAGAAATGGCTGAAGGAAAGCCCCCTTATGCTGATAT
CCATCCAATGAGGGCAATCTTCATGATTCCTACAAATCCTCCTCCCACA
TTCCGAAAACCAGAGCTATGGTCAGATAACTTTACAGATTTTGTGAAAC
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AGCAGGAGATTGAAGAGATCCGGCAGAAGTACCAGTCCAAGCGGCAGCC
CATCCTGGATGCCATAGAGGCTAAGAAGAGACGGCAACAAAACTTCtga
gcggccgc
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