University of Dundee

Standard Operating Procedure

Preparation of active MST2 [2 - 491]

Enzyme description:- MST2 [2 - 491]

Clone number:- DU 1433

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 82, 940.58 daltons
Average Mass 82, 994.06 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.27

Purity:- > 85 %

Activation protocol:- Constitutively active

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 [Long term stability to be determined]

Assay:- Standard filter binding assay

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

Substrate:-
Myelin Basic Protein. Final concentration: 0.3 mg/ml

Specific activity range:- To be determined

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CLONE DATA SHEET - MST2 [2 - 491]

Protein MST2 [2 - 491]

Clone number DU 1433

Species Human

Accession number U60206

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG
DKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIA
DKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSK
DFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVT
HPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEA
IPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLE
VLFQGPLG**SEQPPAPKSKLKKLSEDSLTKQPEEVFDV
LEKLGEGSYGSVFKAIHKESGQVVAIKQVPVESDLQ
EIIKEISIMQQCDSPYVVKYYGSYFKNTDLWIVMEY
CGAGSVSDIIRLRNKTLIEDEIATILKSTLKGLEYLHF
MRKIHRDIKAGNILLNTEGHAKLADFGVAGQLTD
TMAKRNTVIGTPFWMAPEVIQEIGYNCVADIWSLG
ITSIEMAEGKPPYADIHPMRAIFMIPTNPPPTFRKPEL
WSDDFTDFVKKCLVKNPEQRATATQLLQHPFIKNA
KPVSILRDLITEAMEIKAKRHEEQQRELEEEEENSDE
DELDSHTMVKTSVESVGTMRATSTMSEGAQTMIE
HNSTMLESDLGTMVINSEDEEEEDGTMKRNATSPQ
VQRPSFMDYFDKQDFKNKSHENCNQNMHEPFPMS
KNVFPDNWKVPQDGDFDFLKNLSLEELQMRLKAL
DPMMEREIEELRQRYTAKRQPILDAMDAKKRRQQ
NF**

Native sequence Amino acids E2 – F491 (end) of human MST2.
Residue E232 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Bam*H1 and *Eco*R1 site in pFastBAC GST

Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGG
CCTTGTGCAACCCACTCGACTTCTTTTGGAATATCT
TGAAGAAAAATATGAAGAGCATTTGTATGAGCGC
GATGAAGGTGATAAATGGCGAAACAAAAAGTTTG
AATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATA
TTGATGGTGATGTTAAATTAACACAGTCTATGGCC
ATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAA
TGCTTGAAGGAGCGGTTTTGGATATTAGATACGGT
GTTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAAT
GCTGAAAATGTTCGAAGATCGTTTATGTCATAAAA
CATATTTAAATGGTGATCATGTAACCCATCCTGACT
TCATGTTGTATGACGCTCTTGATGTTGTTTTATACAT
GGACCCAATGTGCCTGGATGCGTTCCCAAAATTAG
TTTGTTTTAAAAAACGTATTGAAGCTATCCCACAA
ATTGATAAGTACTTGAAATCCAGCAAGTATATAGC
ATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTG
GTGGCGACCATCCTCCAAAATCGGATCTGGAAGTT
CTGTTCCAGGGGCCCCTGGGATCC**GAGCAGCCGC**
CGGCGCCTAAGAGTAAACTAAAAAAGCTGAGTG
AAGACAGTTTGACTAAGCAGCCTGAAGAAGTTT
TTGATGTATTAGAGAAGCTTGGAGAAGGGTCTT
ATGGAAGTGTATTTAAAGCAATACACAAGGAAT
CCGGTCAAGTTGTCGCAATTAAACAAGTACCTGT
TGAATCAGATCTTCAGGAAATAATCAAAGAAAT
TTCCATAATGCAGCAATGTGACAGCCCATATGTT
GTAAAGTACTATGGCAGTTATTTTAAGAATACA
GACCTCTGGATTGTTATGGAGTACTGTGGCGCT
GGCTCTGTCTCAGACATAATTAGATTACGAAACA
AGACATTAATAGAAGATGAAATTGCAACCATTC
TTAAATCTACATTGAAAGGACTAGAATATTTGCA
CTTTATGAGAAAAATACACAGAGATATAAAAGC
TGGAAATATTCTCCTCAATACAGAAGGACATGC
AAAATTGGCAGATTTTGGAGTGGCTGGTCAGTT
AACAGATACAATGGCAAAACGCAATACTGTAAT
AGGAACTCCATTTTGGATGGCTCCTGAGGTGAT
TCAAGAAATAGGCTATAACTGTGTGGCCGACAT
CTGGTCCCTTGGCATTACTTCTATAGAAATGGCT
GAAGGAAAACCTCCTTATGCTGATATACATCCAA
TGAGGGCTATTTTTATGATTCCCACAAATCCACC
ACCAACATTCAGAAAGCCAGAACTTTGGTCCGA

TGATTTCACCGATTTTGTTAAAAAGTGTTTGGTG
AAGAATCCTGAGCAGAGAGCTACTGCAACACAA
CTTTTACAGCATCCTTTTATCAAGAATGCCAAAC
CTGTATCAATATTAAGAGACCTGATCACAGAAG
CTATGGAGATCAAAGCTAAAAGACATGAGGAAC
AGCAACGAGAATTGGAAGAGGAAGAAGAAAAT
TCGGATGAAGATGAGCTGGATTCCCACACCATG
GTGAAGACTAGTGTGGAGAGTGTGGGCACCAT
GCGGGCCACAAGCACGATGAGTGAAGGGGCCC
AGACCATGATTGAACATAATAGCACGATGTTGG
AATCCGACTTGGGGACCATGGTGATAAACAGTG
AGGATGAGGAAGAAGAAGATGGAACTATGAAA
AGAAATGCAACCTCACCACAAGTACAAAGACCA
TCTTTCATGGACTACTTTGATAAGCAAGACTTCA
AGAATAAGAGTCACGAAAACTGTAATCAGAACA
TGCATGAACCCTTCCCTATGTCCAAAAACGTTTT
TCCTGATAACTGGAAAGTTCCTCAAGATGGAGA
CTTTGACTTTTTGAAAAATCTAAGTTTAGAAGAA
CTACAGATGCGGTTAAAAGCACTGGACCCCATG
ATGGAACGGGAGATAGAAGAACTTCGTCAGAG
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GATGGATGCAAAGAAAAGAAGGCAGCAAAACT
TTTGA