

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active MST2 [2 - 491]**

|                                     |                                      |
|-------------------------------------|--------------------------------------|
| <b><u>Enzyme description:-</u></b>  | MST2 [2 - 491]                       |
| <b><u>Clone number:-</u></b>        | DU 1433                              |
| <b><u>Source:-</u></b>              | Recombinant                          |
| <b><u>Expression system:-</u></b>   | Baculovirus expression vector system |
| <b><u>Tag:-</u></b>                 | N-terminal GST                       |
| <b><u>Purification method:-</u></b> | GSH Sepharose                        |
| <b><u>Expression level:-</u></b>    | 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]**

|                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Protein</u></b>                       | MST2 [2 - 491]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b><u>Clone number</u></b>                  | DU 1433                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b><u>Species</u></b>                       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b><u>Accession number</u></b>              | U60206                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b><u>Tags</u></b>                          | N-terminal GST                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b><u>Baculovirus expressed protein</u></b> | <p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG<br/>LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA<br/>VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH<br/>VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS<br/>KYIAWPLQGWQATFGGGDHPPKSDLEVLFGGPLGSE<b>QPPAPKSKLKKLS</b><br/><b>EDSLTKQPEEVFDVLEKLGE</b>SGSYGSVFKAIHKESGQVVAIKQVPVESDL<br/>QEIIKEISIMQQCDSPYVVKYYGSYFKNTDLWIVMEYCGAGSVSDIIRL<br/>RNKTLIEDEIATILKSTLKGLEYLHFMRKIHRDIKAGNILLNTEGHAKL<br/>ADFGVAGQLTDTMAKRNTVIGTPFWMAPEVIQEIGYNCVADIWSLGITS<br/>IEMAEGKPPYADIHMPRAIFMIPTNPPPTFRKPELWSDDFTDFVKKCLV<br/>KNPEQRATATQLLQHPFIKNAKPVSI LRDLITEAMEIKAKRHEEQREL<br/>EEEEENSDEDELDSHTMVKTSVESVGTMRATSTMSEGAQTMIEHNSTML<br/>ESDLGTMVINSEDEEEEDGTMKR NATSPQVQRPSFMDYFDKQDFKNKSH<br/>ENCNQNMHEFPMSKNVFPDNWKVPQDGFDFLKNLSLEELQMRLKALD<br/>PMMEREIEELRQRYTAKRQPI LDAMDAKKRRQQNF</p> |
| <b><u>Native sequence</u></b>               | <p>Amino acids E2 – F491 (end) of human MST2.<br/>Residue E232 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b><u>Protease cleavage</u></b>             | PreScission ( <u>LEVLFQGPL</u> ) residues 221 - 229                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b><u>Cloning sites</u></b>                 | <i>Bam</i> H1 and <i>Eco</i> R1 site in pFastBAC GST                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

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### Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA  
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTGTGA  
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT  
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA  
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAACATGTT  
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG  
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT  
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA  
AATGTTTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT  
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT  
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA  
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC  
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGTTG  
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCT  
GGGATCCGAGCAGCCGCCGGCGCCTAAGAGTAAACTAAAAAGCTGAGT  
GAAGACAGTTTGACTAAGCAGCCTGAAGAAGTTTTTGTATGATTAGAGA  
AGCTTGGAGAAGGGTCTTATGGAAGTGATTTAAAGCAATACACAAGGA  
ATCCGGTCAAGTTGTCGCAATTAACAAGTACCTGTTGAATCAGATCTT  
CAGGAAATAATCAAAGAAATTTCCATAATGCAGCAATGTGACAGCCCAT  
ATGTTGTAAAGTACTATGGCAGTTATTTTAAGAATACAGACCTCTGGAT  
TGTTATGGAGTACTGTGGCGCTGGCTCTGTCTCAGACATAATTAGATTA  
CGAAACAAGACATTAATAGAAGATGAAATTGCAACCATTCTTAAATCTA  
CATTGAAAGGACTAGAATATTTGCACTTTATGAGAAAAATACACAGAGA  
TATAAAAGCTGGAAATATTCTCCTCAATACAGAAGGACATGCAAAATTG  
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GGGCTATTTTTATGATTCCACAAATCCACCACCAACATTCAGAAAGCC  
AGAACTTTGGTCCGATGATTTACCGATTTTGTAAAAAGTGTTTGGTG  
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TTATCAAGAATGCCAAACCTGTATCAATATTAAGAGACCTGATCACAGA  
AGCTATGGAGATCAAAGCTAAAAGACATGAGGAACAGCAACGAGAATTG  
GAAGAGGAAGAAGAAAATTCCGATGAAGATGAGCTGGATTCCACACCA  
TGGTGAAGACTAGTGTGGAGAGTGTGGGCACCATGCGGGCCACAAGCAC  
GATGAGTGAAGGGGCCCAGACCATGATTGAACATAATAGCACGATGTTG  
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CCCATGATGGAACGGGAGATAGAAGAACTTCGTGAGAGATACACTGCGA  
AAAGACAGCCCATCTGGATGCGATGGATGCAAGAAAAAGAAGGCAGCA  
AAACTTTtga