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Standard Operating Procedure

Preparation of active MST4 [1 - 416]

<u>Enzyme description:-</u>	MST4 [1 - 416]
<u>Clone number:-</u>	DU 8430
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	4 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	49, 869.02 daltons
Average Mass	49, 900.44 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.3
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine	
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
Myelin Basic Protein	Final concentration: 0.3 mg/ml
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

MST4 [1 - 416]

Protein MST4 [1 – 416]

Clone number DU 8430

Species Human

Accession number NM_016542.3

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFOGAMGSM**MAHSPVAVQVPGM**QNNIADP
EELFTKLERIGKGSFGEVFKGIDNRTQQVVAIKIIDLEEADEIEDIQ
QEITVLSQCDSSYVTKYYSYLKGSKLWIIMEYLGGSALDLLRAGPF
DEFQIATMLKEILKGLDYLHSEKKIHRDIKAANVLLSEQGDVKLADFG
VAGQLTDTQIKRNTFVGTPFWMAPEVIQQSAYDSKADIWSLGITAIEL
AKGEPNDSMHPMRVLFLIPKNNPPTLVGDFTKSFKEFIDACLNKDPS
FRPTAKELLKHKFIVKNSKKTSYLTELIDRFKRWKAEGHSDDESSEG
SDSESTSRENNTHPEWSFTTVRKKPDPKQVQNGAEQDLVQTLSCLSMI
ITPAFAELKQODENNASRNQAIEELEKSIAVAEAACPGITDKMVKKL
EKFQKCSADESP

Native sequence Amino acids M1 – P416 (end) of human MST4.
Residue M29 of the fusion protein is equivalent to M1 of MST4.
The His(6) tag is located at residues 5 - 10 of the fusion protein.

Protease cleavage rTEV (ENLYFOG) residues 18 - 24

Cloning sites *Bam*H1 and *Not*1 of pFastBac HTb

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**Nucleotide
sequence of insert**

ggatccATGGCCCACTCGCCGGTGGCTGTCCAAGTGCCTGGGATGCAG
AATAACATAGCTGATCCAGAAGAACTGTTACAAAATTAGAGCGCATT
GGGAAAGGCTCATTTGGGGAAGTTTTCAAAGGAATTGATAACCGTACC
CAGCAAGTCGTTGCTATTTAAAATCATAGACCTTGAGGAAGCCGAAGAT
GAAATAGAAGACATTCAGCAAGAAATAACTGTCTTGAGTCAATGTGAC
AGCTCATATGTAACAAAATACTATGGGTCATATTTAAAGGGGTCTAAA
TTATGGATAATAATGGAATACCTGGGCGGTGGTTCAGCACTGGATCTT
CTTCGAGCTGGTCCATTTGATGAGTTCCAGATTGCTACCATGCTAAAG
GAAATTTTAAAAGGTCTGGACTATCTGCATTCAGAAAAGAAAATTCAC
CGAGACATAAAAAGCTGCCAATGTCTTGCTCTCAGAACAAGGAGATGTT
AAACTTGCTGATTTTGGAGTTGCTGGTCAGCTGACAGATACACAGATT
AAAAGAAATACCTTTGTGGGAACTCCATTTTGGATGGCTCCTGAAGTT
ATTCAACAGTCAGCTTATGACTCAAAGCTGACATTTGGTCATTGGGA
ATTACTGCTATTGAACTAGCCAAGGGAGAGCCACCTAACTCCGATATG
CATCCAATGAGAGTTCTGTTTTCTTATTCCCAAAAACAATCCTCCAAC
CTTGTTGGAGACTTTACTAAGTCTTTTAAGGAGTTTATTGATGCTTGC
CTGAACAAAGATCCATCATTTTCGTCCTACAGCAAAGAAGCTTCTGAAA
CACAAATTCATTGTAAAAAATTCAAAGAAGACTTCTTATCTGACTGAA
CTGATAGATCGTTTTAAGAGATGGAAGGCAGAAGGACACAGTGATGAT
GAATCTGATTCCGAGGGCTCTGATTCCGGAATCTACCAGCAGGGAAAAC
AATACTCATCCTGAATGGAGCTTTACCACCGTACGAAAGAAGCCTGAT
CCAAAGAAAGTACAGAATGGGGCAGAGCAAGATCTTGTGCAAACCCTG
AGTTGTTTGTCTATGATAATCACACCTGCATTTGCTGAACTTAAACAG
CAGGACGAGAATAACGCTAGCAGGAATCAGGCGATTGAAGAAGCTCGAG
AAAAGTATTGCTGTGGCTGAAGCCGCCTGTCCCGGCATCACAGATAAA
ATGGTGAAGAACTAATTGAAAAATTTCAAAGTGTTTCAGCAGACGAA
TCCCCCtaagcggccgc