

Division of Signal Transduction Therapy

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_013254

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGSMASHPVAVQVPGMNNIADP
EELFTKLERIGKGSFGEVFKGIDNRTQQVVAIKIIDLEEADEIEDIQ
QEITVLSQCDSSYVTKYYGSYLKGSKLWIIMEYLGGSALDLLRAGPF
DEFQIATMLKEILKGLDYHSEKKIHRDIKAANVLLSEQGDVKLADFG
VAGQLTDTQIKRNTFVGTPFWMAPEVIQQSAYDSKADIWSLGITAIEL
AKGEPNDSMHMPMRVLFLLPKNNPPTLVGDFTKSFKEFIDACLNKDPS
FRPTAKELLKHKFIVKNSKKTSYLTTELIDRFKRWKAEGHSDDSDSEG
SDSESTSRENNTHPEWSFTTVRKKPDPKKVQNGAEQDLVQTLSCLSMI
ITPAFAELKQODENNASRNQAIEELEKSIAVAEEACPGITDKMVKKLI
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 (ENLYFQG) residues 18 - 24

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

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Nucleotide sequence of insert

ggatccATGGCCCACTCGCCGGTGGCTGTCCAAGTGCCTGGGATGCAG
AATAACATAGCTGATCCAGAAGAACTGTTACAAAATTAGAGCGCATT
GGGAAAGGCTCATTTGGGGAAGTTTTCAAAGGAATTGATAACCGTACC
CAGCAAGTCGTTGCTATTAAAATCATAGACCTTGAGGAAGCCGAAGAT
GAAATAGAAGACATTCAGCAAGAAATAACTGTCTTGAGTCAATGTGAC
AGCTCATATGTAACAAAATACTATGGGTCATATTTAAAGGGGTCTAAA
TTATGGATAATAATGGAATACCTGGGCGGTGGTTCAGCACTGGATCTT
CTTCGAGCTGGTCCATTTGATGAGTTCCAGATTGCTACCATGCTAAAG
GAAATTTTAAAAGGTCTGGACTATCTGCATTTCAGAAAAGAAAATTCAC
CGAGACATAAAAGCTGCCAATGTCTTGCTCTCAGAACAGGAGATGTT
AAACTTGCTGATTTTGGAGTTGCTGGTCAGCTGACAGATACACAGATT
AAAAGAAATACCTTTGTGGGAAGTCCATTTTGGATGGCTCCTGAAGTT
ATTCAACAGTCAGCTTATGACTCAAAGCTGACATTTGGTCATTGGGA
ATTACTGCTATTGAACTAGCCAAGGGAGAGCCACCTAACTCCGATATG
CATCCAATGAGAGTTCTGTTTCTTATTCCCAAAAACAATCCTCCAAC
CTTGTTGGAGACTTTACTAAGTCTTTTAAGGAGTTTATTGATGCTTGC
CTGAACAAAGATCCATCATTTTCGTCCTACAGCAAAAGAACTTCTGAAA
CACAAATTCATTGTAAAAAATTCAAAGAAGACTTCTTATCTGACTGAA
CTGATAGATCGTTTTAAGAGATGGAAGGCAGAAGGACACAGTGATGAT
GAATCTGATTCCGAGGGCTCTGATTCTGGAATCTACCAGCAGGGAAAAC
AATACTCATCCTGAATGGAGCTTTACCACCGTACGAAAGAAGCCTGAT
CCAAAGAAAGTACAGAATGGGGCAGAGCAAGATCTTGTCGAAACCCTG
AGTTGTTTGTCTATGATAATCACACCTGCATTTGCTGAACTTAAACAG
CAGGACGAGAATAACGCTAGCAGGAATCAGGCGATTGAAGAAGTTCGAG
AAAAGTATTGCTGTGGCTGAAGCCGCCTGTCCCGGCATCACAGATAAA
ATGGTGAAGAACTAATTGAAAAATTTCAAAGTGTTTCAGCAGACGAA
TCCCCCtaagcggccgc