Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MST3 [19 – 289]

Enzyme description:-	MST3 [19 - 289]
Clone number:-	DU 62777
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal His(6)
Purification method:-	Cobalt Agarose

Calculated molecular mass:-

Monoisotopic	33, 199.24 daltons
Average Mass	33, 220.06 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	5.67
Purity:-	85 %
Activation protocol:-	Constitutively active

Enzyme storage buffer:-
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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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

Substrate:-
Myelin Basic Protein (MBP)
Final concentration: 0.33 mg/ml

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Clone Data Sheet

MST3 [19 - 289]

Protein	MST3 [19 - 289]
Clone number	DU 62777
Species	Human
Accession number	Q9Y6E0-2
Tags	N-terminal His6
Bacterially expressed protein	`MGSSHHHHHHSSGLEVLFQGPGSAM`**DPEELFTKLEKIGKGSFGEVFKGIDNRTQKVVAIKIIDLEEAEDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTKLWIIMEYLGGGSALDLLEPGPLDETQIATILREILKGLDYLHSEKKIHRDIKAANVLLSEHGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGITAIELARGEPPHSELHPMKVLFLIPKNNPPTLEGNYSKPLKEFVEACLNKEPSFRPTAKELLKHKFILRNAKKTSYLTELID**
Native sequence	Amino acids D19 – D289 (end) of human MST3. Residue D26 of the fusion protein is equivalent to D19 of the native enzyme. The His6 tag is located at residues 5 – 10.
Protease cleavage	PreScission (LEVLFQGP) residues 14 - 21
Cloning sites	*Bam*H1 and *Not*1 sites of pET15b

Nucleotide Sequence of insert

ggatccgccatgGACCCAGAAGAGCTTTTTACAAAACTAGAGAAAATTG
GGAAGGGCTCCTTTGGAGAGGTGTTCAAAGGCATTGACAATCGGACTCA
GAAAGTGGTTGCCATAAAGATCATTGATCTGGAAGAAGCTGAAGATGAG
ATAGAGGACATTCAACAAGAAATCACAGTGCTGAGTCAGTGTGACAGTC
CATATGTAACCAAATATTATGGATCCTATCTGAAGGATACAAAATTATG
GATAATAATGGAATATCTTGGTGGAGGCTCCGCACTAGATCTATTAGAA
CCTGGCCCATTAGATGAAACCCAGATCGCTACTATATTAAGAGAAATAC
TGAAAGGACTCGATTATCTCCATTCGGAGAAGAAAATCCACAGAGACAT
TAAAGCGGCCAACGTCCTGCTGTCTGAGCATGGCGAGGTGAAGCTGGCG
GACTTTGGCGTGGCTGGCCAGCTGACAGACACCCAGATCAAAAGGAACA
CCTTCGTGGGCACCCCATTCTGGATGGCACCCGAGGTCATCAAACAGTC
GGCCTATGACTCGAAGGCAGACATCTGGTCCCTGGGCATAACAGCTATT
GAACTTGCAAGAGGGGAACCACCTCATTCCGAGCTGCACCCCATGAAAG
TTTTATTCCTCATTCCAAAGAACAACCCACCGACGTTGGAAGGAAACTA
CAGTAAACCCCTCAAGGAGTTTGTGGAGGCCTGTTTGAATAAGGAGCCG
AGCTTTAGACCCACTGCTAAGGAGTTATTGAAGCACAAGTTTATACTAC
GCAATGCAAAGAAAACTTCCTACTTGACCGAGCTCATCGACtagcggcc
gc