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Certificate of Analysis

MST1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-624, 14-624-K, 14-624M
Parent Lot # 1643507

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, full-length, human MST1, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 88.7% by SDS-PAGE and Coomassie blue staining. MW = 57kDa.

Specific Activity (Parent lot# 1643507): 645U/mg, where one unit of MST1, active activity is defined as 1nmol phosphate incorporated into 250µM (KKSRGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.607mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

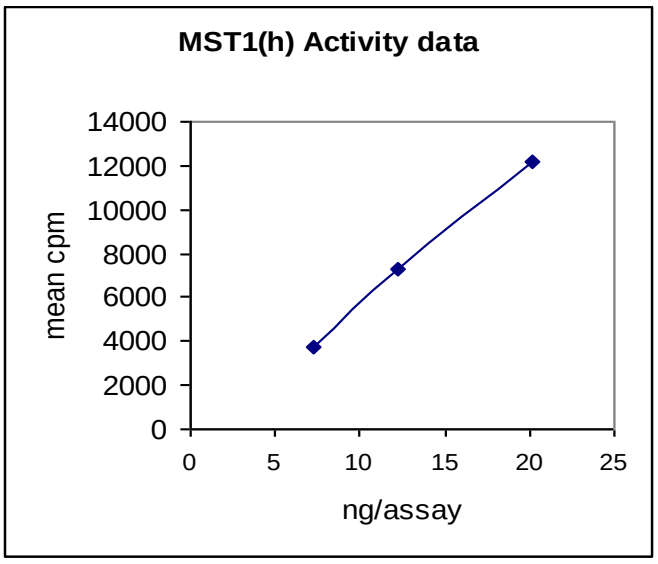
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

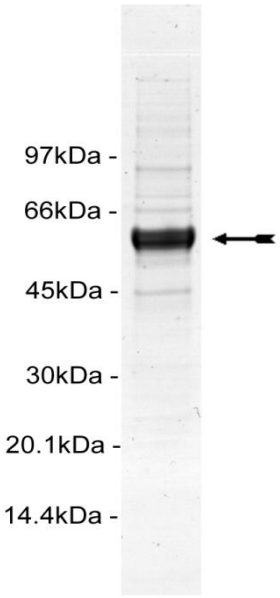
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 7.3–20.2ng of this lot of enzyme phosphorylated 250µM (KKSRGDYMTMQIG) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as MST1 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MST1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKSRGDYMTMQIG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **MST1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7.3–20.2ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure:

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKSRGDYMTMQIG)**.
3. Add **2.5µl (7.3–20.2ng) MST1, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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MST1 Sequence Information

Protein human MST1

Tags *N*-terminal 6His

Native sequence M10 of the recombinant sequence is equivalent to M1 of native human MST1

Accession number GenBank NM_006282

Recombinant MST1 amino acid sequence:

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   1 MHHHHHHEFM ETVQLRNPPR RQLKKLDEDS LTKQPEEVFD VLEKLGEGSY GSVYKAIHKE
  61 TGQIVAIKQV PVESDLQEII KEISIMQQCD SPHVVKYYGS YFKNTDLWIV MEYCGAGSVS
 121 DIIRLRNKTL TEDEIATILQ STLKGLEYLH FMRKIHRDIK AGNILLNTEG HAKLADFGVA
 181 GQLTDTMAKR NTVIGTPFWM APEVIQEIGY NCVADIWSLG ITAIEMAEGK PPYADIHPMR
 241 AIFMIPTNPP PTFRKPELWS DNFTDFVKQC LVKSPEQRAT ATQLLQHPFV RSAKGVSILR
 301 DLINEAMDVK LKRQESQQRE VDQDDEENSE EDEMDSGTMV RAVGDEMGTV RVASTMTDGA
 361 NTMIEHDDTL PSQLGTMVIN AEDEEEEGTM KRRDETMQPA KPSFLEYFEQ KEKENQINSF
 421 GKSVPGPLKN SSDWKIPQDG DYEFLKSWTV EDLQKRLLAL DPMMEQEIEE IRQKYQSKRQ
 481 PILDAIEAKK RRQQNF
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Recombinant MST1 nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcatg gagacggtac agctgaggaa cccgccgcgc
  61 cggcagctga aaaagttgga tgaagatagt ttaaccaaac aaccagaaga agtatttgat
 121 gtcttagaga aacttggaga agggtcctat ggcagcgtat acaaagctat tcataaagag
 181 accggccaga ttgttgctat taagcaagtt cctgtggaat cagacctcca ggagataatc
 241 aaagaaatct ctataatgca gcaatgtgac agccctcatg tagtcaaata ttatggcagt
 301 tattttaaga acacagactt atggatcgtt atggagtact gtggggctgg ttctgtatct
 361 gatatcattc gattacgaaa taaaacgtta acagaagatg aaatagctac aatattacaa
 421 tcaactctta agggacttga ataccttcat tttatgagaa aaatacaccg agatatcaag
 481 gcaggaaata ttttgctaaa tacagaagga catgcaaaac ttgcagattt tggggtagca
 541 ggtcaactta cagataccat ggccaagcgg aatacagtga taggaacacc attttggatg
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 661 ataactgcca tagaaatggc tgaaggaaag ccccttatg ctgatatcca tccaatgagg
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1081 aatactatga ttgagcacga tgacacgttg ccatcacaac tgggcaccat ggtgatcaat
1141 gcagaggatg aggaagagga aggaactatg aaaagaaggg atgagaccat gcagcctgcg
1201 aaaccatcct ttcttgaata ttttgaacaa aaagaaaagg aaaaccagat caacagcttt
1261 ggcaagagtg tacctggtcc actgaaaaat tcttcagatt ggaaaatacc acaggatgga
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1381 gaccccatga tggagcagga gattgaagag atccggcaga agtaccagtc caagcggcag
1441 cccatcctgg atgccataga ggctaagaag agacggcaac aaaacttctg a
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Reviewed and approved by site quality representative.