

Certificate of Analysis

MST2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-524, 14-524-K, 14-524M

Parent Lot # 30895U

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 MST2 residues 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 57.2kDa.

Specific Activity (Parent lot# 30895U): 543U/mg, where one unit of MST2, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.00mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

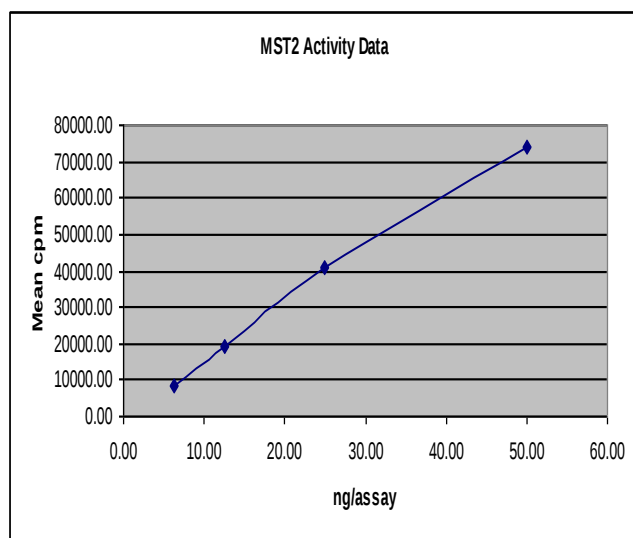
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

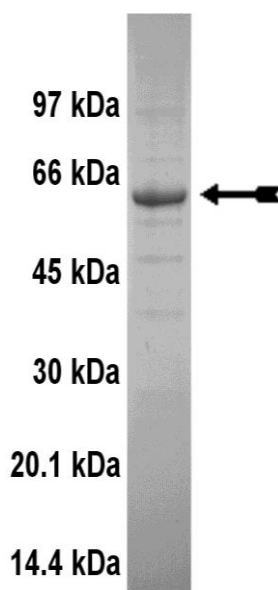
Quality Control Testing

Kinase Assay: 6.25–50.0ng of this lot of enzyme phosphorylated 0.33mg/ml MBP 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 product identity as MST2 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 MST2, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.3mg/ml stock and add 2.5µl of stock per assay point.
3. **MST2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6.25–50.0ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer to wells.
2. Add 2.5µl of **MBP**.
3. Add **2.5µl (6.25–50.0ng) MST2, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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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MST2 Sequence Information

Protein	human MST2 (2–end)
Tags	N-terminal 6His
Native sequence	E8 of the recombinant protein is equivalent to E2 of human MST2
Accession number	GenBank U60206

Recombinant MST2 amino acid sequence:

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1  MHHHHHHEQP  PAPKSKLKKL  SEDSLTKQPE  EVFDVLEKLG  EGSYGSVFKA  IHKESGQVVA
61  IKQVPVESDL  QEIIKEISIM  QQCDSPYVVK  YYGSYFKNTD  LWIVMEYCGA  GSVSDIIRLR
121 NKTLEDEIA  TILKSTLKGL  EYLHFMRKIH  RDIKAGNILL  NTEGHAKLAD  FGVAGQLTDT
181 MAKRNTVIGT  PFWMAPEVIQ  EIGYNCVADI  WSLGITSIEM  AEGKPPYADI  HPMRAIFMIP
241 TNPPPTFRKP  ELWSDDFTFD  VKKCLVKNPE  QRATATQLLQ  HPFIKNAKPV  SILRDLITEA
301 MEIKAKRHEE  QQRELEEEEE  NSDEDELDSE  TMVKTSVESV  GTMRATSTMS  EGAQTMIEHN
361 STMLESDLGT  MVINSEDEEE  EDGTMKRNAT  SPQVQRPSFM  DYFDKQDFKN  KSHENCNQNM
421 HEPFPMKSNV  FPDNWKVPQD  GDFDFLKNLS  LEELQMRLKA  LDPMMEREIE  ELRQRYTAKR
481 QPILDAMDAK  KRRQQQNF

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Recombinant MST2 nucleotide sequence:

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1  atgcatcacc  atcaccatca  tgagcagccg  ccggcgccta  agagtaaact  aaaaaagctg
61  agtgaagaca  gtttgactaa  gcagcctgaa  gaagtttttg  atgtattaga  gaagcttgga
121  gaagggtcct  atggaagtgt  atttaaagca  atacacaagg  aatccgggtc  agttgtcgca
181  attaaacaag  tacctgttga  atcagatcct  caggaaataa  tcaaagaaat  ttccataatg
241  cagcaatgtg  acagcccata  tgttgtaaa  tactatggca  gttattttta  gaatacagac
301  ctctggattg  ttatggagta  ctgtggcgct  ggctctgtct  cagacataat  tagattacga
361  aacaagacat  taatagaaga  tgaaattgca  accattctta  aatctacatt  gaaaggacta
421  gaatatattg  actttatgag  aaaaatacac  agagatataa  aagctggaaa  tattctcctc
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541  atggcaaaac  gcaatactgt  aataggaact  ccattttgga  tggctcctga  ggtgattcaa
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1321  ggagactttg  actttttgaa  aaatctaagt  ttagaagaac  tacagatgcg  gttaaaagca
1381  ctggacccca  tgatggaacg  ggagatagaa  gaacttcgtc  agagatacac  tgcgaaaaga
1441  cagcccattc  tggatgcatg  ggatgcaaag  aaaagaaggc  agcaaaactt  ttga

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