

MST3, Active

Recombinant protein expressed in Sf9 cells

Catalog # S42-11G

Lot # P180-2

Product Description

Recombinant human MST3 (1-311) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_001032296](#).

Gene Aliases

STK24, MST-3, STK3, MST3B, RP11-111L24.5

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

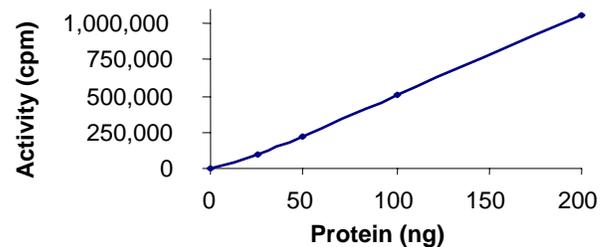
MST3 or Mammalian Sterile 20-like kinase 3 is a member of the germinal center kinase-III family. MST3 contains a conserved kinase domain at its NH(2)-terminus and a regulatory domain at its COOH-terminus. Caspase-mediated cleavage of the regulatory domain of MST3 activates its intrinsic kinase activity and leads to nuclear translocation. Expression of COOH-terminal truncated MST3 in cells results in DNA fragmentation and induction of apoptosis (1). MST3 can inhibit cell migration in a fashion dependent on autophosphorylation and can regulate paxillin phosphorylation through tyrosine phosphatase PTP-PEST (2).

References

- Huang, C Y. et al: Caspase activation of mammalian sterile 20-like kinase 3 (Mst3). Nuclear translocation and induction of apoptosis. J Biol Chem. 2002 Sep 13;277(37):34367-74.
- Lu, T J.: Inhibition of Cell Migration by Autophosphorylated Mammalian Sterile 20-Like Kinase 3 (MST3) Involves Paxillin and Protein-tyrosine Phosphatase-PEST. J Biol Chem. 2006 Dec 15;281(50):38405-17.

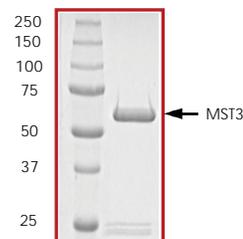
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Specific Activity



The specific activity of MST3 was determined to be **265 nmol /min/mg** as per activity assay protocol.

Purity



The purity of MST3 was determined to be **>90%** by densitometry, approx. MW **63kDa**.

MST3, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	S42-11G
Specific Activity	265 nmol/min/mg
Specific Lot Number	P180-2
Purity	>90%
Concentration	0.1 $\mu\text{g}/\mu\text{l}$
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: S42-11G)

Active MST3 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active MST3 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: P15-58)

PKCtide synthetic peptide substrate (ERM₁PRK₂QGSVRRRV) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active MST3, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active MST3 (Catalog #S42-11G)
 - Component 2.** 10µl of 1mg/ml stock solution of substrate (Catalog #P15-58)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³²P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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