Store at -80°C

#7638

# Mst4 Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

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**Description:** Purified recombinant full length human Mst4 kinase, supplied as a GST fusion protein.

**Background:** Mammalian Ste20-like kinases, including Mst1-4, belong to the germinal center kinase (GCK) family. The amino-terminal kinase domain of Mst is considerably homologous to the kinase domain of yeast Ste20 kinase and other p21-activated kinases (1). The carboxy-terminal region of Mst1 and Mst2 contains a dimerization and an inhibitory domain (1-3). Dimerization and phosphorylation at the activation loop regulates translocation of Mst1 from the cytosol to the nucleus (3). Growing evidence indicates that Mst1, Mst2 and Mst3 are activated by apoptotic signals as well as other stress conditions (4-6). The full activation of Mst1 requires both phosphorylation and caspase-mediated cleavage (4). Sequence alignment of the activation loop of the GCK family indicates that Thr183 of Mst1 and Thr180 of Mst2 are the conserved residues and might be critical for the activity of the kinases. Activated Mst kinases may rely on p38 MAPK and JNK pathways to amplify apoptotic signals (5). Phosphorylation at Ser327 of Mst1, which is close to the caspase-3 recognization site, inhibits caspase-mediated cleavage (4).

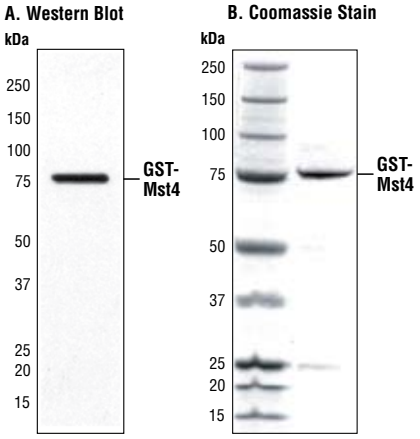


*Figure 1. The purity of the Mst4 fusion protein was analyzed using SDS/PAGE followed by anti-GST Western blot (A) or Coomassie stain (B).*

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length Mst4 (Met1-Pro416) (GenBank Accession No. NM-016542) with an amino-terminal GST tag. The protein was then purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the GST-Mst4 kinase fusion protein is 76 kDa. The purified kinase fusion protein was quality controlled for purity using SDS-PAGE followed by Coomassie stain and Western blot [Fig.1]. Mst4 kinase activity was determined in radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure Mst4 activity using HTScan™ Mst4 Kinase Assay Kit #7639 [Fig.3].

**Background References:**

(1) Dan, I. et al. (2001) *Trends Cell Biol.* 11, 220–230.

(2) Creasy, C.L. et al. (1996) *J. Biol. Chem.* 271, 21049–21053.

(3) Lee, K. and Yonehara, S. (2002) *J. Biol. Chem.* 277, 12351–12358.

(4) Graves, J.D. et al. (2001) *J. Biol. Chem.* 276, 14909–14915.

(5) Lee, K. et al. (2001) *J. Biol. Chem.* 276, 19276–19285.

(6) Graves, J.D. et al. (1998) *EMBO J.* 17, 2224–2234.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

**Companion Products:**

HTScan™ Mst4 Kinase Assay Kit #7639

Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide #1344

Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

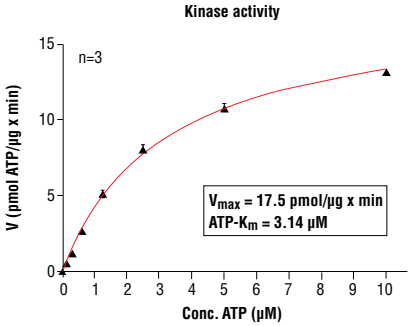


*Figure 2. Mst4 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 uM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: Myelin basic protein, 5 µg/50 µl, Recombinant Mst4: 100 ng/50 µl.*

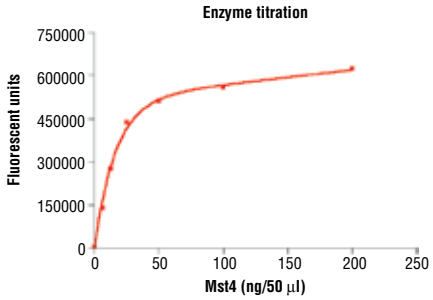


*Figure 3. Dose dependence curve of Mst4 kinase activity: DELFIA® data generated using Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141 to detect phosphorylation of substrate peptide (#1344) by Mst4 kinase. In a 50 µl reaction, increasing amounts of Mst4 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

# Protocol for Mst4 Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

## Kinase

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

## A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide #1344
8. DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

*DELFIA® is a registered trademark of PerkinElmer Life Sciences*

## B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with $dH_20$ to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM $Na_3VO_4$, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml $dH_20$ to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

**Final Assay Conditions for a 50 µl Reaction**

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM $Na_3VO_4$
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng Mst4 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl $dH_2O$/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    **Please note:** This protocol was validated using a Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide and Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com