Store at -80°C

#7639

HTScan® Mst4 Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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Products Included	Products #	Kit Quantity
Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody	3141	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide	1344	1.25 ml
Mst4 Kinase (recombinant, human)	7638	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant active human Mst4 kinase. It includes active Mst4 kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine/threonine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: YKT*LR

Molecular Weights: Peptide substrate, Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide #1344: 1,958 Daltons, GST-Mst4 Kinase: 76 kDa.

Background: Mst kinases, members of the STE20 family of kinases, are upstream activators of MAPK pathways that regulate processes such as apoptosis, morphogenesis and cytoskeletal rearrangements.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 dimerization and inhibitory domains (1-3). Dimerization and phosphorylation at the activation loop results in 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). Complete 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).

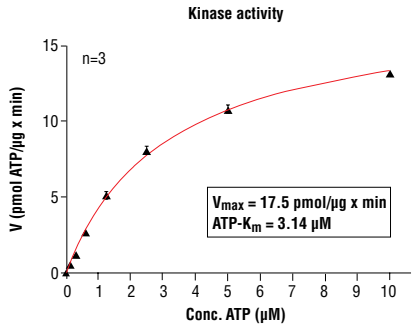

Figure 1. Mst4 kinase activity was measured in a radioisotopic filter binding assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20.000, Substrate: Myelin basic protein, 5 µg/50 µl, recombinant Mst4: 100 ng/50 µl.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human 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 substrate peptide was selected using our Serine/Threonine Kinase Substrate Screening Kit #7400. Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141 was used for detection. The quality of the biotinylated peptides was evaluated by reverse-phase HPLC and by mass spectrometry.

The specific activity of the Mst4 kinase was determined using a radiometric assay [Fig.1].Time course [Fig.2], kinase dose-dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify Mst4 activity using the Mst4 substrate peptide provided in this kit. Mst4 sensitivity to the inhibitor staurosporine was measured using the Mst4 substrate peptide provided in this kit [Fig.5].

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Serine/Threonine Kinase Substrate Screening Kit #7400

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

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

Mst4 Kinase #7638

Staurosporine #9953

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.

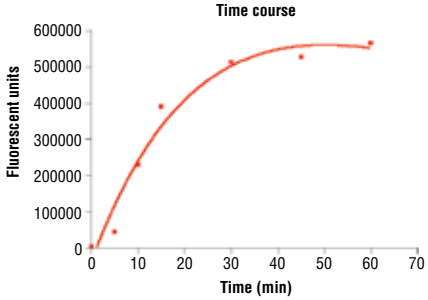

Figure 2. Time course of Mst4 kinase activity: DELFIA® data generated using Phospho-Ezrin (Thr567)/Radixin (Thr564)/ Moesin (Thr558) Antibody #3141 to detect phosphorylation of Mst4 substrate peptide (#1344) by Mst4 kinase. In a 50 µl reaction, 50 ng Mst4 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

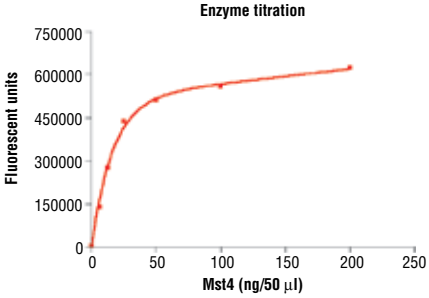

Figure 3. Dose dependence curve of Mst4 kinase activity: DELFIA® data generated using Phospho-Ezrin (Thr567)/ Radixin (Thr564)/Moesin (Thr558) Antibody #3141 to detect phosphorylation of substrate peptide (#1344) by Mst4 kinase. In a 50 µl reaction, increasing amounts of Mst4 and 1.5 µM substrate peptide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

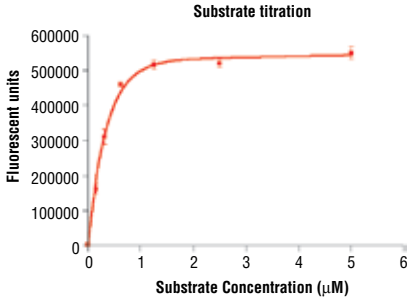

Figure 4. Peptide concentration dependence of Mst4 kinase activity: DELFIA® data generated using Phospho-Ezrin (Thr567)/ Radixin (Thr564)/Moesin (Thr558) Antibody #3141 to detect phosphorylation of substrate peptide (#1344) by Mst4 kinase. In a 50 µl reaction, 50 ng of Mst4 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

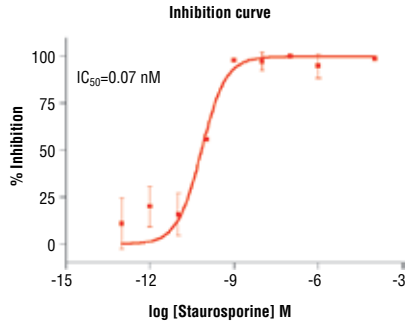

Figure 5. Staurosporine inhibition of Mst4 kinase activity: DELFIA® data generated using Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141 to detect phosphorylation of Mst4 substrate peptide (#1344) by Mst4 kinase. In a 50 µl reaction, 50 ng Mst4, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® Mst4 Kinase Assay Kit

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

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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 [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. Transfer 1.2 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 4 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, Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

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

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