

Mst2 Kinase

✓ 5 µg



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Description: Purified recombinant full length human Mst2 (Met1-Phe491) 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 recognition site, inhibits caspase-mediated cleavage (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Mst2 (Met1-Phe491) (GenBank Accession No. BC010640) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

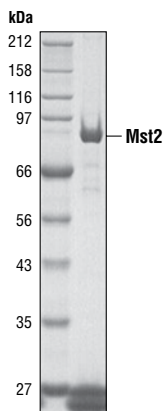


Figure 1. The purity of the GST-Mst2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-Mst2 fusion protein is 83 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Mst2 kinase activity was determined using a radiometric assay [Fig.2].

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.

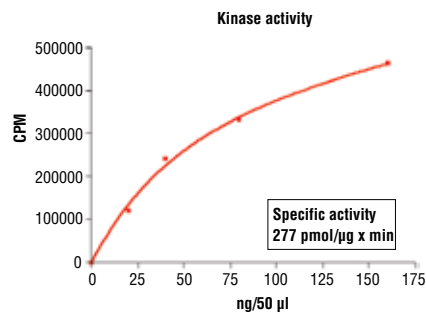


Figure 2. Mst2 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT, 40 ng/µL BSA, 50 µM ATP, 0.6 µM BSA, Substrate: MBP 400 ng/µL and recombinant Mst2: variable.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Protocol for Mst2 Kinase Assay

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. Kinase Buffer (10X)**
40 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM $MgCl_2$
0.5 mM DTT
6 μ M BSA
- 2.** ATP (10 mM) #9804
- 3.** ^{32}P - γ ATP
- 4.** MBP

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute Mst2 protein (100 ng/ μ l concentration) to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted Mst2 kinase solution, 10 μ l MBP (1 μ g/ μ l) and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 4 mM MOPS, pH 7.2
 - 2.5 mM β -glycerophosphate
 - 1 mM EGTA
 - 0.4 mM EDTA
 - 4 mM $MgCl_2$
 - 0.05 mM DTT
 - 0.6 μ M BSA
 - 400 ng/ μ l MBP
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

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