Store at -80°C

#7442

# MAPKAPK-2 Kinase

☑5 µg

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**Description:** Purified recombinant human MAPKAPK-2 (Phe46-His400) kinase, supplied as a GST fusion protein.

**Background:** In response to cytokines, stress and chemotactic factors, MAP kinase-activated protein kinase 2 (MAPKAPK-2) is rapidly phosphorylated and activated. It has been shown that MAPKAPK-2 is a direct target of p38 MAPK (1). Multiple residues of MAPKAPK-2 are phosphorylated *in vivo* in response to stress. However, only four of the residues: Thr25, Thr222, Ser272 and Thr334 are phosphorylated by p38 MAPK in an *in vitro* kinase assay (2). Phosphorylation at Thr222, Ser272 and Thr334 appears to be essential for the activity of MAPKAPK-2 (2). Thr25 is phosphorylated by p42 MAPK *in vitro*, but is not required for the activation of MAPKAPK-2 (2).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human MAPKAPK-2 (Phe46-His400) (GenBank Accession No. NM_032960) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

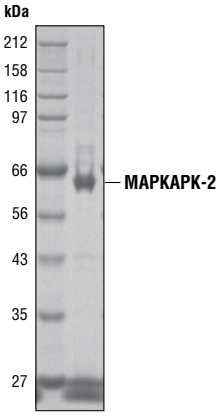


*Figure 1. The purity of the GST-MAPKAPK-2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.*

**Quality Control:** The theoretical molecular weight of the GST- MAPKAPK-2 fusion protein is 68 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. MAPKAPK-2 kinase activity was determined using a radiometric assay [Fig.2].

**Background References:**

(1) Rouse, J. et al. (1994) *Cell* 78, 1027–1037.

(2) Ben-Levy, R. et al. (1995) *EMBO J.* 14, 5920–5930.

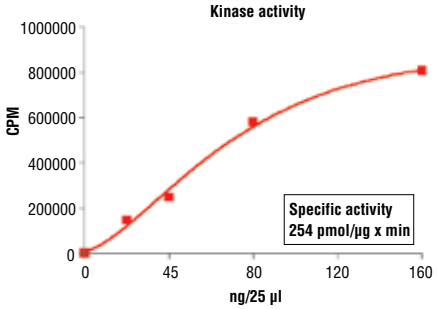


*Figure 2. MAPKAPK-2 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 40 ng/µL BSA, 50 µM ATP, Substrate: MAPKAPK-2 substrate peptide 400 ng/µL and recombinant MAPKAPK-2: 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.

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# Protocol for MAPKAPK-2 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 EDTA
   40 mM $MgC1_2$
   0.5 mM DTT
   6 µM BSA
2. ATP (10 mM) #9804
3. $^{32}$P-γATP
4. MAPKAPK-2 substrate peptide (KKLNRTLSVA) (1µg/µl)

## B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [$^{32}$p] ATP to 0.16 µCi/µl [$^{32}$p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute MAPKAPK-2 protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted MAPKAPK-2 kinase solution, 10 µl MAPKAPK-2 substrate peptide (1 µg/µl) and 5 µl 0.16 µCi/µl [$^{32}$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
40 ng/µl BSA
400 ng/µl MAPKAPK-2 substrate peptide

6. After 15 minutes terminate reaction by spotting 20 µl 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.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.