

MAPKAPK-3 Kinase

✓ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full length human MAPKAPK-3 (Met1-Gln382) kinase, supplied as a GST fusion protein.

Background: MAPKAPK-3 has a single potential SH3-binding site in the proline-rich amino terminus, a putative ATP-binding site, 2 MAP kinase phosphorylation site motifs and a putative nuclear localization signal. It shares 72% nucleotide and 75% amino acid identity with MAPKAPK-2 (1). MAPKAPK-3 has been shown to be activated by growth inducers and stress stimulation of cells. *In vitro* studies demonstrated that ERK, p38 MAP kinase and Jun amino-terminal kinase were all able to phosphorylate and activate MAPKAPK-3, which suggested a role for this kinase as an integrative element of signaling in both mitogen and stress responses (2). This kinase was reported to interact with, phosphorylate and repress the activity of E47, which is a basic helix-loop-helix transcription factor known to be involved in the regulation of tissue-specific gene expression and cell differentiation (3). MAPKAPK-3 may also support luteal maturation through the phosphorylation and activation of the nuclear transcription factor CREB (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human MAPKAPK-3 (Met1-Gln382) (GenBank Accession No. NM_004635) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

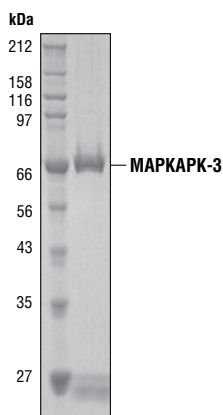


Figure 1. The purity of the GST-MAPKAPK-3 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-MAPKAPK-3 fusion protein is 69 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. MAPKAPK-3 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Sithanandam, G. et al. (1996) *Mol. Cell Biol.* 16, 868–876.
- (2) Ludwig, S. et al. (1996) *Mol. Cell Biol.* 16, 6687–6697.
- (3) Neufeld, B. et al. (2000) *J. Biol. Chem.* 275, 20239–20242.
- (4) Maizels, E.T. et al. (2001) *Mol. Endocrinol.* 15, 716–733.

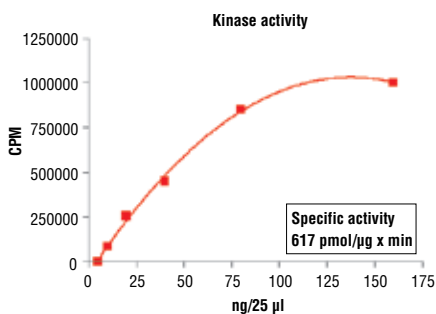


Figure 2. MAPKAPK-3 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 40 ng/ μ l BSA, 50 μ M ATP. Substrate: MBP 800 ng/ μ L and recombinant MAPKAPK-3: variable.

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 MAPKAPK-3 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 $MgCl_2$
0.5 mM DTT
400 ng/ μ l BSA

2. ATP (10 mM) #9804

3. ^{32}P - γ ATP

4. MBP (2 μ g/ μ l)

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 MAPKAPK-3 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 MAPKAPK-3 kinase solution, 10 μ l MBP (2 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
4 mM $MgCl_2$
0.05 mM DTT
40 ng/ μ l BSA
800 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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