

# p38 $\alpha$ MAP Kinase

✓ 5  $\mu$ g



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**Description:** Purified recombinant full length human p38 $\alpha$  MAP Kinase (Met1-Ser360) kinase, supplied as a GST fusion protein.

**Background:** p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase which participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). Four isoforms of p38 MAP kinase,  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  have been identified. Similar to the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors (1-5). MKK3, MKK6 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182. Activated p38 MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 (3) and to phosphorylate the transcription factors ATF-2 (5), Max (6) and MEF2 (5-8).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human p38 $\alpha$  MAP Kinase (Met1-Ser360) (GenBank Accession No. NM\_139012) with an amino-terminal GST tag. The protein was activated by co-expressing with active MKK3 and purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the GST-p38 $\alpha$  MAP Kinase fusion protein is 68 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. p38 $\alpha$  MAP kinase activity was determined using a radiometric assay [Fig.2].

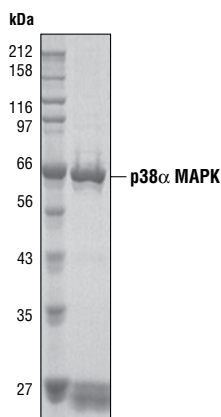


Figure 1. The purity of the GST-p38 $\alpha$  MAP Kinase fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

## Background References:

- (1) Rouse, J. et al. (1994) *Cell* 78, 1027–1037.
- (2) Han, J. et al. (1994) *Science* 265, 808–811.
- (3) Lee, J.C. et al. (1994) *Nature* 372, 739–746.
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- (5) Raingeaud, J. et al. (1995) *J. Biol. Chem.* 270, 7420–7426.
- (6) Zervos, A.S. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 10531–10534.
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- (8) Yang, S.H. et al. (1999) *Mol. Cell. Biol.* 19, 4028–4038.

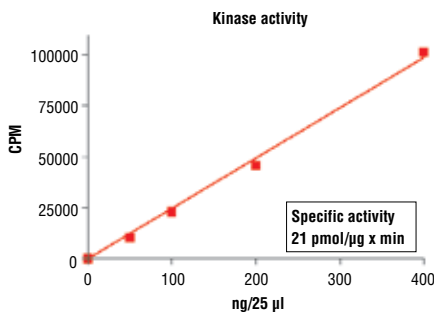


Figure 2. p38 $\alpha$  MAP kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl<sub>2</sub>, 0.05 mM DTT, 40 ng/ $\mu$ l BSA, 50  $\mu$ M ATP. Substrate: MBP 800 ng/ $\mu$ L and recombinant p38 $\alpha$  MAP Kinase: variable.

## Protocol for p38 $\alpha$ MAP 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  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
40 mM  $MgCl_2$   
0.5 mM DTT  
400 ng/ $\mu$ l BSA

2. ATP (10 mM) #9804

3.  $^{32}P$ - $\gamma$ ATP

4. MBP (2  $\mu$ g/ $\mu$ l)

### B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
2. Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute p38 $\alpha$  protein (100 ng/ $\mu$ l concentration) to 40 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10  $\mu$ l diluted p38 $\alpha$  kinase solution, 10  $\mu$ l MBP (2  $\mu$ g/ $\mu$ l) and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

4 mM MOPS, pH 7.2  
2.5 mM  $\beta$ -glycerophosphate  
1 mM EGTA  
4 mM  $MgCl_2$   
0.05 mM DTT  
40 ng/ $\mu$ l BSA  
800 ng/ $\mu$ L MBP

6. After 15 minutes terminate reaction by spotting 20  $\mu$ 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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