

p38 β MAP Kinase

✓ 5 μ g



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Description: Purified recombinant full length human p38 β MAP Kinase (Met1-Gln364) 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, α , β , γ and δ 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 β MAP Kinase (Met1-Gln364) (GenBank Accession No. NM_002751) 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 β 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 β MAP Kinase activity was determined using a radiometric assay [Fig.2].

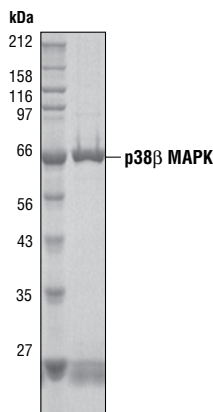


Figure 1. The purity of the GST-p38 β MAP Kinase fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

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.
- (4) Freshney, N.W. et al. (1994) *Cell* 78, 1039–1049.
- (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.
- (7) Zhao, M. et al. (1999) *Mol. Cell. Biol.* 19, 21–30.
- (8) Yang, S.H. et al. (1999) *Mol. Cell. Biol.* 19, 4028–4038.

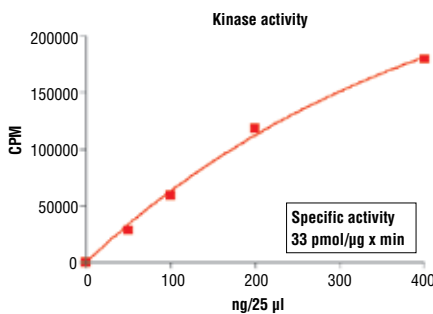


Figure 2. p38 β MAP kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT, 40 ng/ μ l BSA, 50 μ M ATP, Substrate: MBP 800 ng/ μ L and recombinant p38 β MAP Kinase: variable.

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 p38 β 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 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 p38 β protein (100 ng/ μ l concentration) to 50 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 μ l diluted p38 β 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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