

## Product Information

### MKK4, GST-TAGGED

Mouse, Recombinant  
Expressed in *E. coli*

Product Number **M 1689**  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

Synonyms: MAP Kinase Kinase 4, SKK1, MEK4, SEK1, JNKK1

#### Product Description

MKK4, GST-tagged, is produced from a DNA sequence corresponding to amino acids 35-357 of mouse MKK4 containing a glutathione S-transferase (GST) tag at the amino terminus. The recombinant protein is expressed in *E. coli* and is purified by affinity chromatography on glutathione-agarose. MKK4, GST-tagged, is activated by MEKK1 and, thus, is suitable for phosphorylation assays.

The MAP kinase kinases (MAPKKs, MKKs or MEKs) are a family of Thr/Tyr dual specificity protein kinases, upstream of the MAP kinases, that play a central role in mitogenic signaling, transducing extracellular signals to intracellular targets, including transcription factors controlling the expression of genes essential to many cellular processes.<sup>1,2</sup>

MAP Kinase Kinase 4 (MKK4) is a MAPKK isoform distantly related to MAPKK1 (MEK1) that acts within the stress-activated protein kinase pathways.<sup>1-4</sup> MKK4 phosphorylates and activates p38 MAP kinase (SAPK), in response to a variety of stimuli including lipopolysaccharide (LPS) in monocytes, heat shock, UV-irradiation and cytokines (TNF $\alpha$ , IL-1).<sup>4</sup> Activation of c-Jun N-terminal kinase (JNK) in mitogen-stimulated cells is also mediated by MKK4, in a signaling pathway involving MEKK.<sup>5,6</sup> MKK4 activates JNK and p38 MAP Kinase by dual phosphorylation at Thr and Tyr in the Thr-Pro-Tyr and Thr-Gly-Tyr motifs, respectively. Recent studies indicate that MKK4 preferentially phosphorylates the Tyr residue while MKK7 preferentially phosphorylates Thr, and these two kinases may work in concert *in vivo*.<sup>5</sup> In contrast MEK1 prefers the Thr-Glu-Tyr phosphorylation motif of ERKs.<sup>7</sup> MKK4 appears to be widely expressed in many tissues and cells.<sup>5</sup>

MKK4 is activated *in vivo* by different forms of cellular stress and by proinflammatory cytokines. Activation occurs through phosphorylation of Ser and Thr residues at positions 219 and 223, respectively, by MEKK.<sup>6</sup> Activation of MKK4 via the  $\alpha_{1B}$ -adrenergic receptor (a G protein-coupled receptor) appears to require the  $\alpha$ -subunit of Gq, c-Src tyrosine kinase and a Rho family GTPase.<sup>8</sup>

#### Reagent

Mouse recombinant MKK4, GST-tagged, is supplied as a solution in 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35, 1 mM benzamidine, and 0.2 mM phenylmethylsulfonyl fluoride (PMSF).

#### Storage/Stability

The protein is stable for at least six months when stored at  $-70\text{ }^{\circ}\text{C}$ . Centrifuge the original vial after thawing and prior to removing the cap for maximum recovery of product. After initial thawing, store the remaining solution in single-use aliquots at  $-70\text{ }^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Do not store in a frost-free freezer.

#### Product Profile

Purity is approx. 70% by SDS-PAGE with Coomassie blue staining. The major band corresponds to the molecular weight of MKK4, approximately 67 kDa. The preparation may contain several lower molecular weight bands.

One unit of MKK4 activity is defined as the amount of enzyme required to incorporate 1 nmol of phosphate into GST-AFT2 per minute following the activation of JNK1 $_{\alpha 1}$  by MKK4.

## References

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4. Lin, A., et al., Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. *Science*, **268**, 286-290 (1995).
5. Cuenda, A., Mitogen-Activated Protein Kinase Kinase 4 (MKK4). *Int. J. Biochem. Cell Biol.*, **32**, 581-587 (2000).
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7. Minden, A., et al., Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science*, **266**, 1719-1723 (1994).
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