

# MAPK8 (JNK1) [6His-tagged]

Kinase

Alternate Names: Mitogen-Activated Protein Kinase 8, PRKM8, C-Jun Kinase 1, SAPK1, SAPK1c, JNK-46

Cat. No. 66-0028-050

Lot. No. 30307

Quantity: 50 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

## Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. MAP kinases are serine, threonine, and tyrosine specific protein kinases that regulate proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis in response to stimuli, such as mitogens, osmotic stress, heat shock and pro-inflammatory cytokines. Cloning of human Mitogen Activated Protein kinase 8 (MAPK8 or JNK1) was first described by Derijard *et al.* (1994). An example of such interplay between the phosphorylation and ubiquitylation systems has been highlighted in a recent study uncovering a link between the deubiquitylating enzyme CYLD and the oncogene c-MYC, via MAPK8, offering a mechanistic insight into the role of CYLD deficiency in tumour initiation and progression (Panem *et al.*, 2014).

## Physical Characteristics

**Species:** human

**Source:** baculovirus expression vector system

**Quantity:** 50 µg

**Concentration:** 1.27 mg/ml

**Formulation:** 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% β-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

**Molecular Weight:** ~45.1 kDa

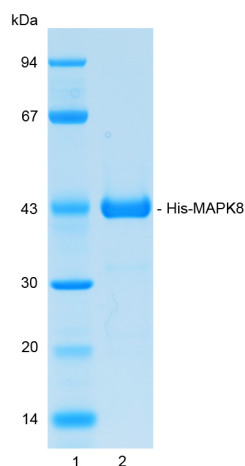
**Purity:** >95% by InstantBlue™ SDS-PAGE

**Stability/Storage:** 12 months at -70°C; aliquot as required

**Protein Sequence:** Please see page 2

## Quality Assurance

**Purity:**  
4-12% gradient SDS-PAGE  
InstantBlue™ staining  
Lane 1: MW markers  
Lane 2: 2.5 µg His-MAPK8



**Protein Identification:**  
Confirmed by mass spectrometry.

**Activity Assay:**  
The specific activity of His-MAPK8 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-MAPK8 was incubated for 10 minutes at 30°C in kinase reaction buffer in the presence of GST-ATF2 [19-96] substrate (0.2 mg/ml) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

**His-MAPK8 specific activity:**  
148.2 Units/mg (188.2 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: GST-ATF2 [residues 19-96]

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Lot-specific COA version tracker: v1.0.0

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## Background

Continued from page 1

### References:

Derijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T *et al.* (1994) JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**, 1025-1037.

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Pannem RR, Dom C, Ahlqvist K, Bosserhoff AK, Hellerbrand C and Massoumi R (2014) CYLD controls c-MYC expression through the JNK-dependent signaling pathway in hepatocellular carcinoma. *Carcinogenesis* **35**, 461-468.

## Physical Characteristics

Continued from page 1

### Protein Sequence:

**MHHHHH**MSRSKRDNNFYSVEIGDSTFTV  
LKRYQNLKPIGSGAOGIVCAAYDAILERN  
VAIKKLSRPFQNTAKRAYRELVLMKCVN  
HKNIIGLLNVFTPKSLEEFQDVYIVMELM  
DANLCQVIQMELDHERMSYLLYQMLCGIKHLH  
SAGIIHRDLKPSNIVVKS DCTLKILDF  
GLARTAGTSFMMPYVTRYRRAPEVIL  
GMGYKENVDLWSVGCIMGEMVCHKILF  
PGRDYIDQWNKVIQLGTPCFEPMKKLQPT  
VRTYVENRPKYAGYSFEKLPDVLFPAD  
SEHNK LKASQARDLLSKMLVIDASKRIS  
VDEALQHPYINWYDPSEAEAPPKIPDKQL  
DEREHTIEEWKELIYKEVMDLEERTKNGVIR  
GQPSPLAQVQQ

Tag (**bold text**): N-terminal 6His  
MAPK8 (regular text): Start **bold italics** (amino acid residues 1-384).

Accession number: AAA36131.1



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Lot-specific COA version tracker: v1.0.0