

MAPK3 (ERK1) [untagged]

Kinase

Alternate Names: Extracellular Signal-Regulated Kinase 1, p44ERK1, p44MAPK

Cat. No. 66-0026-050
Lot. No. 30305

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 3 (MAPK3) was first described by Charest *et al.* (1993) and Garcia *et al.* (1998). Activation of MAPK3 requires both tyrosine and threonine phosphorylation that is mediated by Map/Erk Kinase Kinase 1 (MEK1) (Cobb, *et al.*, 1994). MAPK3 is ubiquitously distributed in tissues with the highest expression in heart, brain and spinal cord (Boulton, *et al.*, 1991). MAPK3 translocates to the nucleus resulting in the phosphorylation of several transcription factors such as Elk-1, c-Myc, c-Jun, c-Fos, and C/EBP beta. MAPK3 has also been shown to interact with several proteins includ-

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.28 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: ~43.2 kDa

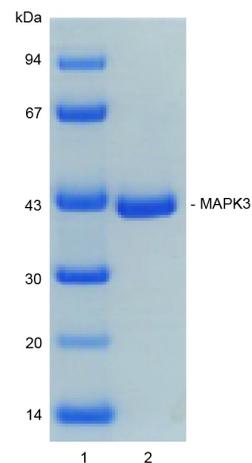
Purity: >95% by InstantBlue™ SDS-PAGE

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 GST-MAPK3



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of MAPK3 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. MAPK3 was incubated for 10 minutes at 30°C in kinase reaction buffer in the presence of MBP substrate (0.333 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.

MAPK3 specific activity:
1299 Units/mg (364 Units/ml)

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

Substrate: Myelin Basic Protein (MBP)



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Lot-specific COA version tracker: v1.0.1

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

ing DUSP3, HDAC4, MAP2K1 and MAP2K2 (Marti, *et al.*, 1997; Todd, *et al.*, 1999; Zhou, *et al.*, 2000).

References:

Boulton TG *et al.* (1991) ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* **65**, 663-675.

Charest DL *et al.* (1993) Molecular cloning, expression, and characterization of the human mitogen-activated protein kinase p44erk1. *Mol Cell Biol* **13**, 4679-4690.

Cobb MH *et al.* (1994) The mitogen-activated protein kinases, ERK1 and ERK2. *Semin Cancer Biol* **5**, 261-268.

Garcia F *et al.* (1998) Molecular cloning and characterization of the human p44 mitogen-activated protein kinase gene. *Genomics* **50**, 69-78.

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

Marti A *et al.* (1997) Actin-binding protein-280 binds the stress-activated protein kinase (SAPK) activator SEK-1 and is required for tumor necrosis factor- α activation of SAPK in melanoma cells. *J Biol Chem* **272**, 2620-2628.

Todd JL, Tanner KG and Denu JM (1999) Extracellular regulated kinases (ERK) 1 and ERK2 are authentic substrates for the dual-specificity protein-tyrosine phosphatase VHR. A novel role in down-regulating the ERK pathway. *J Biol Chem* **274**, 13271-13280.

Zhou X *et al.* (2000) Histone deacetylase 4 associates with extracellular signal-regulated kinases 1 and 2, and its cellular localization is regulated by oncogenic Ras. *Proc Natl Acad Sci U S A* **97**, 14329-14333.

Physical Characteristics

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Protein Sequence:

LGSAAAAAAQQGGGGGEP~~RR~~T~~EG~~V~~GP~~G~~VP~~G~~EV~~E~~VM~~
VKGQPF~~DV~~GPRYTQLQYIGEGAYGMVSSAYDH
VRKTRVAIKKISPF~~EH~~QTYCQRT~~LR~~EIQILL
RFRHENVIGIRDIL~~RA~~STLEAMRDVYIVQDL
METDLYKLLKSQQLSNDHICYFLYQILRGLKY
IHSANVLHRDLKPSNLLSNTTCDLKI~~CD~~F
GLARIADPEHDHTGFLTEYVATRWYRAPEIM
LNSKGYTKSIDIWSVGCILAEMLSNRP~~IF~~PG
KHYLDQLNHILGILGSPSQEDLNCIINMKAR
NYLQSLPSKTKVAWAKLFPKSDSKALDLLDRM
LTFNPNKRI~~TVE~~EALAHPLYEQYDPTDEP
VAEEPFTFAMELDDLPKERL~~KE~~LIFQETAR
FQPGVLEAP

The residues underlined remain after cleavage and removal of the purification tag.

MAPK3 (regular text): Start **bold italics** (amino acid residues 2-379).

Accession number: CAA42744.1



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