MAPK14 (SAPK2A) [GST-tagged]

Kinase

Alternate Names: Cytokine-Suppressive Anti-inflammatory Drug-Binding Protein 1, CSBP1, SAPK2A, p38-Alpha

Cat. No. **66-0034-050**
Lot. No. **30313**

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 14 (MAPK14 or SAPK2A) was first described by Lee *et al.* (1994). An example of such interplay between phosphorylation and ubiquitylation has been highlighted in a recent study providing direct evidence for p38α/p38γ (MAPK14/MAPK11) in mediating oxidative stress-induced autophagy-related genes, suggesting that these MAPKs regulate both the ubiquitin-proteasome and the autophagy-lysosome systems in muscle wasting (McClung *et al.*, 2010).

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 6.06 mg/ml

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

Molecular Weight: ~67.5 kDa

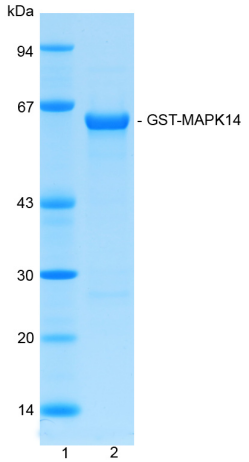
Purity: >95% by InstantBlue™ SDS-PAGE

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-MAPK14



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-MAPK14 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. GST-MAPK14 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.

GST-MAPK14 specific activity:
144.0 Units/mg (872.4 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: v1.0.0

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Background

Continued from page 1

References:

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

Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D *et al.* (1994) A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **372**, 739-746.

McClung JM, Judge AR, Powers SK and Yan Z (2010) p38 MAPK links oxidative stress to autophagy-related gene expression in cachectic muscle wasting. *Am J Physiol Cell Physiol* **298**, C542-549.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLVPRGS***M***SQERPTFYRQELNK**
TIWEVPERYQNLSPVGSGAYGSVCAAFDT
KTGLRVAVKKLSRPFQSIIHAKRTYREL
RLLKHMKHENVIGLLDVFTPARSLEEFND
VYLVTHLMGADLNNIVKCQKLTDDHVQFLIY
QILRGLKYIHSADIIHRDLKPSNLAVNEDCEL
KILDFGLARHTDDEMTGYVATRWYRAPEIM
LNWMHYNQTVDIWSVGCIMAELLTGRTLF
PGTDHIDQLKLILRLVGTPGAELLKKISS
ESARNYIQSLTQMPKMNFANVFIGAN
PLAVDLLEKMLVLDSDKRITAAQALAHAY
FAQYHDPDDEPVADPYDQSFESRDLLIDEWK
SLTYDEVISFVPPPLDQEEMES

Tag (**bold text**): N-terminal GST
Protease cleavage site: Thrombin (LVPR▼GS)
MAPK14 (regular text): Start ***bold italics*** (amino acid residues 1-360).
Accession number: AAA57456.1

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Lot-specific COA version tracker: v1.0.0