MARK4 [6His-tagged]

Kinase

Alternate Names: MAP/Microtubule Affinity-regulating Kinase 4; MARK4; MAP/Microtubule Affinity-regulating Kinase-like 1; MARKL1; KIAA1860

Cat. No. **66-0005-050**
Lot. No. **2134**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background by Sir Philip Cohen

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. Cloning of human Microtubule Affinity Regulating Kinase 4 (MARK4) was first described by Kato *et al.* (2001). MARK4 is a member of the subfamily of protein kinases that include the AMP-activated protein kinase (AMPK) and, like AMPK itself, is activated by the tumour suppressor kinase LKB1 (Lizcano *et al.*, 2004). The physiological roles of MARK4 include the phosphorylation of microtubule associated proteins and the regulation of cell polarity. Members of the MARK sub-family also phosphorylate tau at sites that induce its dissociation from tubulin. Enhanced phosphorylation of these sites is an early hall mark of Alzeheimer's disease and is followed by abnormal aggregation of tau to paired helical filaments that are found in people with Alzheimer's disease (Marx *et al.*, 2010). MARK4 contains a ubiquitin-like domain adjacent to the kinase catalytic domain and undergoes Lys29/

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Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 50 µg

Concentration: 1 mg/ml

Formulation: 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 150 mM NaCl, 270 mM sucrose, 0.03% Brij, 0.1% β-Mercapto-ethanol, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~85.7 kDa

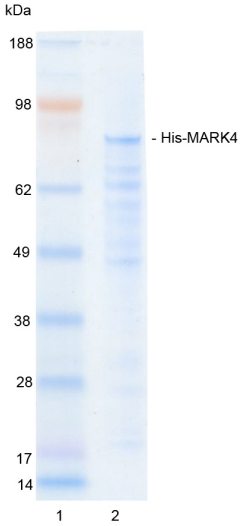
Purity: >40% by InstantBlue™ SDS-PAGE

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: 1 µg His-MARK4



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of His-MARK4 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-MARK4 was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of CHKtide substrate (300 µM) and [γ-^{32}P]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-MARK4 specific activity:
661.7 Units/mg (661.7 Units/ml)

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

Substrate: CHKtide (KKKVSRSGLYRSPSMPENLNRPR)

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Lot-specific COA version tracker: v1.0.0

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Background by Sir Philip Cohen

Continued from page 1

Lys33-linked polyubiquitylation that may inhibit its activity. It also interacts with the deubiquitylase USP9X (Al-Hakim *et al.*, 2008).

References:

Al-Hakim AK, Zagorska A, Chapman L, Deak M, Peggie M, Alessi DR (2008) Control of AMPK-related kinases by USP9X and atypical Lys(29)/Lys(33)-linked polyubiquitin chains. *Biochem J* **411**, 249-60.

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

Kato T, Satoh S, *et al.* (2001) Isolation of a novel human gene, MARKL1, homologous to MARK3 and its involvement in hepatocellular carcinogenesis. *Neoplasia* **3**, 4-9.

Lizcano JM, Goransson O, *et al.* (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* **23**, 833-43.

Marx A, Nugoor C, Panneerselvam S, Mandelkow E (2010) Structure and function of polarity-inducing kinase family MARK/Par-1 within the branch of AMPK/Snf1-related kinases. *FASEB J* **24**, 1637-48.

Background kindly written by:

Sir Philip Cohen FRS, FRSE
University of Dundee

Director of the Medical Research Council Protein Phosphorylation Unit (1990-2012)

Director of the Scottish Institute for Cell Signalling incorporating the Protein Ubiquitylation Unit (2008-2012)

Co-Director of the Division of Signal Transduction Therapy (1998-2012)

Deputy Director of the Division of Signal Transduction Therapy (from July 2012)

Professor Cohen's research group is studying the interplay between protein phosphorylation and protein ubiquitylation in the regulation of innate immunity.

Physical Characteristics

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Protein Sequence:

MSYYHHHHHHDYDIPTTENLYFQGAMGS***S***S
RTVLAPGNDRNSDTHGTLGSGRSSDK
GPSWSSRSLGARCRNSIASCPEEQPH
VGNYRLLRTIGKGNFAKVKLARHILT
GREVAIKIIDKTQLNPSSLQKLFREVRIMK
GLNHPNIVKLFEVIETEKTLYLVMEYASA
GEVFDYLVSHGRMKEKEARAKFRQIVSAVHY
CHQKNIVHRDLKAENLLLDAEANIKIADFGFS
NEFTLGSKLDTFCGSPPYAAPELFQGKKYDG
PEVDIWSLGVILYTLVSGSLPFDGHNLKELR
ERVLRGKYRVPFYMSTDCESILRRFLVLNPAK
RCTLEQIMKDKWINIGYEGEELKPYTEPEED
FGDTKRIEVMVGMGYTREEIKESLTSQKY
NEVTATYLLLGRKTEEGGDRGAPGLA
LARVRAPSDTTNGTSSSKGTSHSK
GQRSSSSTYHRQRRHSDFCGPSPAPLHPKR
SPTSTGEAELKEERLPGRKASCSTAGSGSR
GLPPSSPMVSSAHNPNKAEIPERRKDSTSTP
NNLPPSMMTRRNTYVCTERPGAERPSLLPNG
KENSSGTPRVPPASPSSHSLAPPSGERSR
LARGSTIRSTFHGGQVRDRRAGGGGGGGVQNG
PPASPTLAHEAAPLPAGRPRPTTNLFT
KLTSKLTRRVADEPERIGGPEVTSCHLP
WDQTETAPRLLRFPWSVKLTSSRPPEAL
MAALRQATAAARCRCRQPQPFLLACLHGGAG
GPEPLSHFEVEVCQLPRPGLRGVLFRRVAGTA
LAFRTLVTRISNDLEL

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYF▼QG)
MARK4 (regular text): Start ***bold italics*** (amino acid residues 2-752)
Accession number: NP_001186796.1

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