AMSH-LP CD(264-436) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: KIAA1373 protein, AMSH FP, Associated molecule with the SH3 domain of STAM like protein, STAMBPL1

Cat. No.	**64-0029-050**	Quantity:	50 µg
Lot. No.	**30072**	Storage:	-70˚C

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CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Deconjugating enzymes (DCEs) are proteases that process ubiquitin or ubiquitin-like gene products, reverse the modification of proteins by a single ubiquitin or ubiquitin-like protein (UBL) and remodel polyubiquitin (or poly-UBL) chains on target proteins (Reyes-Turcu *et al.*, 2009). The deubiquitylating – or deubiquitinating – enzymes (DUBs) represent the largest family of DCEs and regulate ubiquitin dependent signalling pathways. The activities of the DUBs include the generation of free ubiquitin from precursor molecules, the recycling of ubiquitin following substrate degradation to maintain cellular ubiquitin homeostasis and the removal of ubiquitin or ubiquitin-like proteins (UBL) modifications through chain editing to rescue proteins from proteasomal degradation or to influence cell signalling events (Komander *et al.*, 2009). There are two main classes of DUB; cysteine proteases and metalloproteases. AMSH-Like Protein (AMSH-LP) is a member of the JAB1/MPN/Mov34 metalloenzyme (JAMM) family and cloning of the human gene was first described by Nagase *et al.* (2000). AMSH and AMSH-LP share 54% identity and 75% sequence similarity in their JAMM domain. It is known that both proteins act as regulators of free ubiquitin in the cell, bind clathrin, contain a putative nuclear localization signal and an MIT domain. However, AMSH-LP lacks some of the key features when compared to AMSH. AMSH contains an SH3-binding motif, which facilitates its interaction with STAM of ESCRT (endosomal sorting complexes required for transport), while a functional SH3-binding motif is lost in AMSH-LP (Davies *et al.*, 2011). AMSH-LP is known to specifically cleave Lys 63-linked polyu-

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~47 kDa

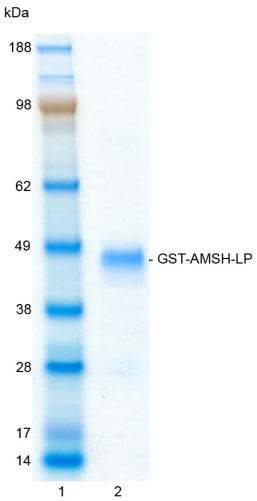
Purity: >92% by InstantBlue™ SDS-PAGE

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 GST-AMSH-LP



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-AMSH-LP was validated by determining the increase in fluorescence measured as a result of the enzyme catalysed cleavage of the fluorogenic substrate Ubiquitin-Rhodamine110-Glycine generating Ubiquitin and Rhodamine110-Glycine. Incubation of the substrate in the presence or absence of GST-AMSH-LP was compared confirming the deubiquitylating activity of GST-AMSH-LP.

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Lot-specific COA version tracker: v1.0.0

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Background

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biquitin chains and does not cleave Lys 48-linked polyubiquitin chains (Sato *et al.*, 2008). After removal of these K63-linked polyubiquitin chains, AMSH-LP can coordinate the recycling of receptors to the cell surface (McCullough *et al.*, 2004). AMSH-LP has been found to be a positive regulator of Tax activation of NF-κB. AMSH-LP indirectly stabilized Tax by promoting its shuttling from the nucleus to the cytoplasm, thereby protecting Tax from K48-induced ubiquitylation and proteasomal degradation in the nucleus. Thus, AMSH-LP is a DUB that controls Tax trafficking in the cell and is essential for exporting Tax from the nucleus to the cytoplasm, where it triggers IKK and NF-κB activation (Lavorgna and Harhaj, 2012).

References:

Davies CW, Paul LN, Kim MI, Das C (2011) Structural and thermodynamic comparison of the catalytic domain of AMSH and AMSH-LP: nearly identical fold but different stability. *J Mol Biol* **413**, 416-429.

Komander D, Clague MJ, Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-563.

Lavorgna A, Harhaj EW (2012) An RNA Interference Screen Identifies the Deubiquitinase STAMBPL1 as a Critical Regulator of Human T-Cell Leukemia Virus Type 1 Tax Nuclear Export and NF-kappaB Activation. *J Virol* **86**, 3357-3369.

McCullough J, Clague MJ, Urbe S (2004) AMSH is an endosome-associated ubiquitin isopeptidase. *J Cell Biol* **166**, 487-492.

Nagase T, Kikuno R, Ishikawa KI, Hirosawa M, Ohara O (2000) Prediction of the coding sequences of unidentified human genes. XVI. The complete sequences of 150 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* **7**, 65-73.

Reyes-Turcu FE, Ventii KH, Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Ann Rev Biochem* **78**, 363-397.

Sato Y, Yoshikawa A, Yamagata A, Mimura H, Yamashita M, Ookata K, Nureki O, Iwai K, Komada M and Fukai S (2008) Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains. *Nature* **455**, 358-362.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA***E***
GLRCVVLPEDLCHKFLQLAESNTVRGI
ETCGILCGKLTHNEFTITHVIVPKQSAGPDY
CDMENVEELFNVQDQHDLLTLGWIHTHPTQTA
FLSSVDLHTHCSYQLMLPEAIAIVCSPKHKDT
GIFRLTNAGMLEVSACKKKGFHPHTKEPRLF
SICKHVLVKDIKIIVLDLR

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
AMSH-LP (regular text): Start ***bold italics*** (amino acid residues 264-436)
Accession number: NP_065850

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