

AMSH CD(252-424) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: STAM binding protein, Associated molecule with the SH3 domain of STAM

Cat. No. 64-0042-050
Lot. No. 30146

Quantity: 50 µg
Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



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 is a member of the JAB1/MPN/Mov34 metalloenzyme (JAMM) family and cloning of the human gene was first described by Tanaka *et al.* (1999). 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, and contain a putative nuclear localization signal and a microtubule interacting and transport (MIT) domain. AMSH also contains a Src homology 3 (SH3) domain, facilitating its interaction with signal transducing adaptor molecule (STAM), while this functional SH3-binding motif is lost in AMSH-LP (Davies, *et al.*, 2011). STAM complexed with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) organises cargo proteins in the multivesicular body (MVB) pathway.

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Physical Characteristics

Species: human

Protein Sequence: Please see page 2

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: ~47.1 kDa

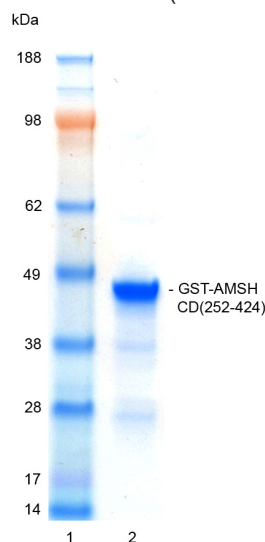
Purity: >89% by InstantBlue™ SDS-PAGE

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

Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-AMSH
CD(252-424)



Protein Identification:

Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:

The activity of GST-AMSH CD(252-424) was validated by the monitoring of mono-ubiquitin generation as a result of the enzyme catalysed cleavage of K63-linked di-ubiquitin. Incubation of the substrate in the presence or absence of GST-AMSH CD(252-424) was compared confirming the deubiquitylating activity of GST-AMSH CD(252-424).



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Lot-specific COA version tracker: v1.0.0

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Background

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AMSH is thought to play a role in the regulation of ubiquitin-mediated degradation by binding to STAM (Kim, *et al.*, 2006). AMSH also plays a significant role in neurodegeneration, demonstrated by AMSH knockout mice exhibiting severe neuronal damage, specifically neuron loss and increasing numbers of apoptotic cells (Suzuki, *et al.*, 2011). AMSH is well known to specifically cleave K63-linked polyubiquitin chains and does not cleave K48-linked polyubiquitin chains (Sato, *et al.*, 2008). After removal of these K63-linked polyubiquitin chains, AMSH can coordinate the recycling of receptors to the cell surface (McCullough, *et al.*, 2004).

References:

Davies, C.W., *et al.* (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.

Kim, M.S., *et al.* (2006) STAM-AMSH interaction facilitates the deubiquitination activity in the C-terminal AMSH, *Biochem Biophys Res Commun* **351**, 612-618.

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

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

Reyes-Turcu, F.E., Ventii, K.H. and Wilkinson, K.D. (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes, *Ann Rev Biochem* **78**, 363-397.

Sato, Y., *et al.* (2008) Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains, *Nature* **455**, 358-362.

Suzuki, S., *et al.* (2011) AMSH is required to degrade ubiquitinated proteins in the central nervous system, *Biochem Biophys Res Commun* **408**, 582-588.

Tanaka, N., *et al.* (1999) Possible involvement of a novel STAM-associated molecule "AMSH" in intracellular signal transduction mediated by cytokines, *J Biol Chem* **274**, 19129-19135.

Physical Characteristics

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MSPILGYWKIKGLVQPTRLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTSMAIRYIADKHNMLGGCP
KERAEISMLEGAVLDIRYGVSRIAYSKD
FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD
HVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPOIDKYLKSSKYIAWPLQG
WQATFGGGDHPKSDLEVLFOGPLGSP
GIPGSTRAAADGLRHVVVPGRLCPQFLQLAS
ANTARGVETCGILCGKLMRNEFTITHVLIP
KQSAGSDYCNTENEEELFLIQDQOGLITLG
WIHTHTPTQTAFLLSSVDLHTHCSYQMMLPES
VAIVCSPKFQETGFFKLTDHGLEEISSCRQKG
FHPHSDPPLFCSCSHVTVVDRAVTITDLR

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFO↓GP)
AMSH CD(252-424) (regular text): Start **bold italics** (amino acid residues 252-424)
Accession number: AAD05037



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