Ataxin-3 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: Machado-Joseph disease protein 1, Spinocerebellar ataxia type 3 protein

Cat. No. **64-0033-050**
Lot. No. **30076**

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. Ataxin-3 is a cysteine protease and is a member of the Machado-Joseph Domain (MJD) enzyme family. Cloning of the human gene was first described by Kawaguchi *et al.* (1994). Machado–Joseph disease (MJD), the most common form of spinocerebellar ataxia worldwide, is a progressive and ultimately fatal neurodegenerative disorder caused by polyQ expansion in ataxin-3, a conserved and ubiquitous protein known to bind polyubiquitin chains and to function as a deubiquitylating enzyme. Ataxin-3 has been linked to protein homeostasis maintenance, transcription, cytoskeleton regulation and myogenesis (Matos *et al.*,

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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: ~69 kDa

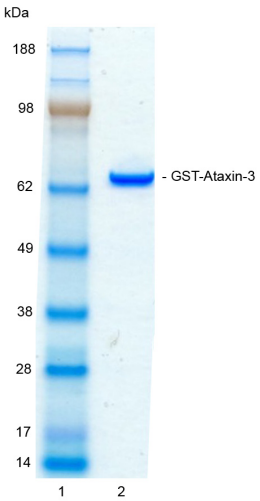
Purity: >98% by InstantBlue™ SDS-PAGE

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-Ataxin-3



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-Ataxin-3 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-Ataxin-3 was compared confirming the deubiquitylating activity of GST-Ataxin-3.

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Lot-specific COA version tracker: v1.0.0

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Background

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2011). Ataxin-3 is an unusual DUB whose properties favour functional interactions with E3s such as Parkin and CHIP (Durcan *et al.*, 2012; Scaglione *et al.*, 2011). Ataxin-3 contains an amino-terminal protease domain followed by three Ubiquitin Interacting Motifs (UIMs) that bind longer ubiquitin chains. Ataxin-3 also trims longer chains, displaying little activity against chains of four or fewer ubiquitins (Scaglione *et al.*, 2011). The full-length protein has been shown to preferentially cleave Lys-63-linked and mixed-linkage ubiquitin chains. This specificity is imparted by UIMs found in the enzyme C-terminal region, as the isolated Josephin domain cleaves both Lys-48 and Lys-63-linked chains with equal efficiency. Ataxin-3 can also cleave adducts at the C terminus of NEDD8, a protein that is closely related to ubiquitin in both structure and sequence (Weeks *et al.*, 2011).

References:

Durcan TM, Kontogiannea M, Bedard N, Wing SS, Fon EA (2012) Ataxin-3 deubiquitination is coupled to Parkin ubiquitination via E2 ubiquitin-conjugating enzyme. *J Biol Chem* **287**, 531-541.

Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I and et al. (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet* **8**, 221-228.

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

Matos CA, de Macedo-Ribeiro S and Carvalho AL (2011) Polyglutamine diseases: the special case of ataxin-3 and Machado-Joseph disease. *Prog Neurobiol* **95**, 26-48.

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

Scaglione KM, Zavodszky E, Todi SV, Patury S, Xu P, Rodriguez-Lebron E, Fischer S, Konen J, Djarmati A, Peng J, Gestwicki JE and Paulson HL (2011) Ube2w and ataxin-3 coordinately regulate the ubiquitin ligase CHIP. *Mol Cell* **43**, 599-612.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCP
KERAEISMLEGAVLDIRYGVSRIAYSKD
FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD
HVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAW
PLQGWQATFGGGDHPPKSDLEVLFQG
PLGS***M***ESIFHEKQEGSLCAQHCLNN
LLQGEYFSPVELSSIAHQLDEEERMR
MAEGGVTSEDYRTFLQQPSGNMDDSGFF
SIQVISNALKVWGLELILFNSPEYQRL
RIDPINERSFICNYKEHWFTVRKLGKQWF
NLNSLLTGPELISDTYLALFLAQLQQE
GYSIFVVKGDLPDCEADQLLQMIRVQQMHRP
KLIGEELAQLKEQRVHKTDLERVLEAN
DGSGMLDEDEEDLQRALALSRQEID
MEDEEADLRRAIQLSMQGSSRNISQD
MTQTSGTNLTSEELRKRREAY
FEKQQQKQQQQQQQQQQQQQQQQQQQGDLS
GQSSHPCERPATSSGALGSDLGDAMSEEDM
LQAAVTMSLETVRNDLKTEGKK

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
Ataxin 3 (regular text): Start ***bold italics*** (amino acid residues 1-370)
Accession number: AAH33711

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