

Ataxin-3L [6His-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Name: Machado-Joseph disease protein 1-like

Cat. No. **64-0034-050**
Lot. No. **30077**

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-3L is a cysteine protease and a member of the Machado-Joseph Domain (MJD) enzyme family. Cloning of the human gene was first described by Gerhard *et al.* (2004). 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

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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: ~43 kDa

Purity: >92% by InstantBlue™ SDS-PAGE

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

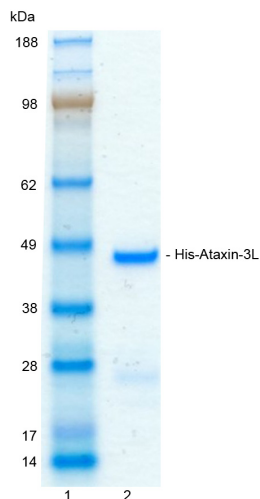
Protein Sequence:

MGSSHHHHHSSGLEVLFQGP~~GS~~MDFI F
HEKQEGFLCAQHC~~LNNLLQGEYFSPVELA~~
SIAHQ~~LDEEERMMAEGGV~~TSE~~EYLAFLQ~~QPS~~EN~~
MDDT~~GFFSIQVISNALKFWGLEIIHFNNPEY~~
QKLGIDPINERS~~FCNYKQHWFTIRKFGKHWFN~~
LNSLLAGPELISDTCLANFLARL~~QQQAYSVFV~~
VKGDL~~PDCEADQLLQIISVEEMDTPKLNK~~
KLVKQKEHRVYKTVLEKVSEES~~DES~~TS~~DQDEED~~
F~~Q~~RALELSRQETNREDEHLRSTIELSMQ~~QSS~~
GNTS~~QDLPKTSCVTPASEQPKKIKEDY~~
FEKHQ~~QEQKQQQQSDLP~~GHSSYLHERPTTSS
RAIESDLSDDISEGTVQAAVD~~TILEIMRKNL~~KIK
GEK

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVL~~FQ~~GP)
Ataxin-3L (regular text): Start **bold italics** (amino acid residues 1-355)
Accession number: NP_001129467

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-Ataxin-3L



Protein Identification:

Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:

The activity of His-Ataxin-3L 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 His-Ataxin-3L was compared confirming the deubiquitylating activity of His-Ataxin-3L.



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Lot-specific COA version tracker: v1.0.0

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Background

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maintenance, transcription, cytoskeleton regulation and myogenesis (Matos *et al.*, 2011). Ataxin-3L shares 85% sequence identity with ataxin-3 although it has recently been shown that the Josephin domain of ataxin-3L demonstrates substantially higher deubiquitylating activity than the ataxin-3 Josephin domain (Weeks *et al.*, 2011).

References:

Gerhard DS, Wagner L, Feingold EA, Shenmen CM, Grouse LH, Schuler G, *et al.* (2004) The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC). *Genome Res* **14**, 2121-2127.

Komander D, Clague MJ, 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, 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, Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Ann Rev Biol* **78**, 363-397.

Weeks SD, Grasty KC, Hernandez-Cuevas L, Loll PJ (2011) Crystal structure of a Josephin-ubiquitin complex: evolutionary restraints on ataxin-3 deubiquitinating activity. *J Biol Chem* **286**, 4555-4565.



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