OTUD6A [6His-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: DUBA-2, DUBA2, OTU domain containing 6A, HSHIN6, FLJ25831, HIN-6 protease

Cat. No. **64-0038-050**
Lot. No. **30133**

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. OTUD6A is a cysteine protease and a member of the OTU (ovarian tumour) superfamily of proteins (Balakirev *et al.,* 2003). Cloning of the human gene was first described by Kayagaki *et al.* (2007). OTU enzymes play important roles as negative-feedback regulators in NF-κB signalling, interferon signalling and in p97 (cdc48)-mediated processes although the cellular functions of most OTU enzymes remain to be discovered. Ovarian tumour family DUBs contain a papain-like catalytic core of ~180 amino acids. In addition to their catalytic

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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: ~35.8 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

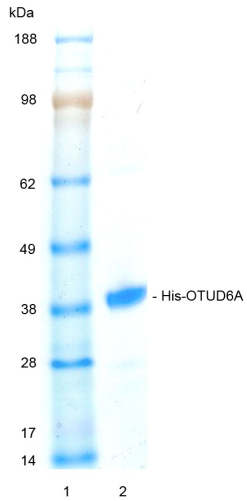
Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPGS***M***DDPK
SEQQRILRRHQRERQELQAQIRSLKNSVPK
TDKTKRKQLLQDVARMEAEMAQKHRQELEK
FQDDSSIESVVEDLAKMNLENRP
PRSSKAHRKRERMESEERERQESIFQAEMSE
HLAGFKREEEEKLAAILGARGLEMKAIPADGH
CMYRAIQDQLVFSVSVEMLRCRTASYMKKH
VDEFLPFFSNPETSDSFGYDDFMIYCDNIVRT
TAWGGQLELRALSHVLKTPIEVIQADSPTLII
GEEYVKKPIILVYLRYAYSLGEHYNSVTPLEA
GAAGGVLPRLL

Tag **(bold text)**: N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
OTUD6A (regular text): Start ***bold italics*** (amino acid residues 1-288)
Accession number: NP_997203

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-OTUD6A



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of His-OTUD6A 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-OTUD6A was compared confirming the deubiquitylating activity of His-OTUD6A.

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Lot-specific COA version tracker: v1.0.0

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Background

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domain, many OTU members have additional ubiquitin-binding domains (UBDs). At least 20 different UBD families have been described, and knowledge of linkage-specific UBDs have provided the means to understand the roles of different ubiquitin linkages in cells (Licchesi *et al.,* 2012).

References:

Balakirev MY, Tcherniuk SO, Jaquinod M and Chroboczek J (2003) Otubains: a new family of cysteine proteases in the ubiquitin pathway. *EMBO Rep* **4**, 517-522.

Kayagaki N, Phung Q, Chan S, Chaudhari R, Quan C, O'Rourke KM, Eby M, Pietras E, Cheng G, Bazan JF, Zhang Z, Arnott D and Dixit VM (2007) DUBA: a deubiquitinase that regulates type I interferon production. *Science* **318**, 1628-1632.

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.

Licchesi JD, Mieszczanek J, Mevissen TE, Rutherford TJ, Akutsu M, Virdee S, El Oualid F, Chin JW, Ovaa H, Bienz M and Komander D (2012) An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. *Nat Struct Mol Biol* **19**, 62-71.

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

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