

# OTUD1 CD(270-481) [6His-tagged]

## Deconjugating Enzyme

Alternate Names: OTU domain-containing protein 1, DUBA-7, OTUD1, DUBA7, OTDC1

Cat. No. 64-0054-050

Lot. No. 30234

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. OTUD1 is a cysteine protease and is a member of the OTU 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 domain, many OTU members have additional ubiquitin-binding domains (UBDs). At

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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:** ~30 kDa

**Purity:** >98% by InstantBlue™ SDS-PAGE

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

### Protein Sequence:

**MGSSHHHHHSSG**LEVLFGPGSPF  
PGVEAPSSAAEPVIVSRSDPRDEKLALY  
LAEVEKQDKYLQRNRYRFHIIPDGN  
CLYRAVSKTVYGDQSLHRELREQTVHYIAD  
HLDHFSPLEIGDVGFEFIIAAAQD  
GAWAGYPELLAMGQMLNVNIHLTTG  
GRLESPTVSTMIHYLGPEDSLRPSI  
WLSWLSNGHYDAVFDHSYPNPEYDNWCK  
QTQVQRKRDEELAKSMAISLSKMYIEQNAC  
SZLDSKAAADPDPAAANKAPKRKVG F

Tag (**bold text**): N-terminal 6His

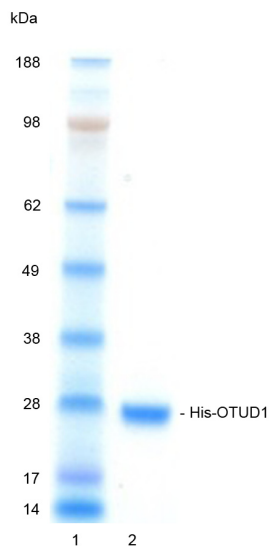
Protease cleavage site: PreScission™ (LEVLFG▼GP)

OTUD1 (regular text): Start **bold italics** (amino acid residues 270-481)

Accession number: NP\_001138845

## Quality Assurance

**Purity:** 4-12% gradient SDS-PAGE InstantBlue™ staining  
Lane 1: MW markers  
Lane 2: 1 µg His-OTUD1



### Protein Identification:

Confirmed by mass spectrometry.

### Deubiquitylase Enzyme Assay:

The activity of His-OTUD1 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-OTUD1 was compared confirming the deubiquitylating activity of His-OTUD1.



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Lot-specific COA version tracker: v1.0.0

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## Background

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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). OTUD1 has recently been shown to have K63-linkage specificity. Studies undertaken to investigate this phenomenon have proven that it is the C-terminal ubiquitin-interacting motif (UIM, residues 457–476 of OTUD1) that provides its efficiency and specificity for K63-linkages (Mevisen *et al.*, 2013).

### 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, et al. (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, Mevisen TE, Rutherford TJ, Akutsu M, Virdee S, et al. (2012) An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. *Nature Structural & Molecular Biology* 19, 62-71.

Mevisen TE, Hospenthal MK, Geurink PP, Elliott PR, Akutsu M, Arnaudo N, Ekkebus R, Kulathu Y, Wauer T, El Oualid F, Freund SM, Ovaa H, Komander D (2013) OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell* 154, 169-84.

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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