

# OTUB1 [GST-tagged]

## Deconjugating enzyme: Deubiquitylase

**Alternate Names:** FLJ20113, HSPC263, OTB1, OTU domain containing ubiquitin aldehyde binding protein 1, Ubiquitin specific protease otubain 1, Ubiquitin thiolesterase protein OTUB1

**Cat. No.** 64-0011-050  
**Lot. No.** 30147

**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. OTUB1 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 Balakirev *et al.* (2003). OTU 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 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

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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:** ~58.1 kDa

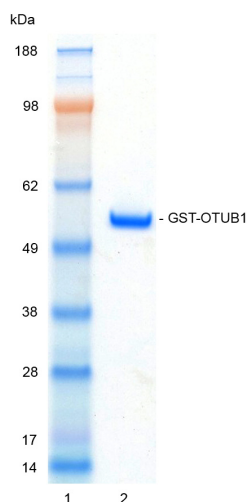
**Purity:** >98% by InstantBlue™ SDS-PAGE

**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-OTUB1



**Protein Identification:**  
Confirmed by mass spectrometry.

**Deubiquitylase Enzyme Assay:**  
The activity of GST-OTUB1 was validated by the monitoring of mono-ubiquitin generation as a result of the enzyme catalysed cleavage of K48-linked di-ubiquitin. Incubation of the substrate in the presence or absence of GST-OTUB1 was compared confirming the deubiquitylating activity of GST-OTUB1.



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Lot-specific COA version tracker: v1.0.0

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## Background

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cells (Licchesi *et al.*, 2012). OTUB1 is highly selective for the cleavage of K48-linked ubiquitin chains and proteomic analyses have indicated that OTUB1 binds to E2s of the UBE2D and UBE2E families including UBE2D1 (Juang *et al.*, 2012). OTUB1 was recently shown to modulate p53 stability through inhibition of UBE2D1. p53 is known to be ubiquitylated and destabilized by MDM2 and several other ubiquitin E3s and both deubiquitylated and stabilized by USP7 and USP10. Recent studies have shown that OTUB1 can directly suppress MDM2-mediated p53 ubiquitylation in cells and *in vitro*. Overexpression of OTUB1 drastically stabilizes and activates p53, leading to apoptosis and marked inhibition of cell proliferation in a p53-dependent manner (Sun *et al.*, 2012). OTUB1 has also been shown to bind to and inhibit UBE2N, the cognate E2 enzyme for the E3 ligase RNF168. OTUB1 can suppress RNF168-dependent poly-ubiquitylation independently of its catalytic activity. OTUB1 depletion mitigates the double strand break repair defect associated with defective Ataxia telangiectasia mutated (ATM) signaling, indicating that pharmacological targeting of the OTUB1-UBE2N interaction might enhance the DNA damage response (Blackford and Stewart, 2011; Nakada *et al.*, 2010).

### 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.

Blackford AN and Stewart GS (2011) When cleavage is not attractive: non-catalytic inhibition of ubiquitin chains at DNA double-strand breaks by OTUB1. *DNA Repair* 10, 245-249.

Juang YC, Landry MC, Sanches M, Vittal V, Leung CC, Ceccarelli DF, *et al.* (2012) OTUB1 co-opts Lys48-linked ubiquitin recognition to suppress E2 enzyme function. *Mol Cell* 45, 384-397.

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, Mieszczynek 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.

Nakada S, Tai I, Panier S, Al-Hakim A, Iemura S, Juang YC, *et al.* (2010) Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. *Nature* 466, 941-946.

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

Sun XX, Challagundla KB and Dai MS (2012) Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. *EMBO J* 31, 576-592.

## Physical Characteristics

Continued from page 1

### Protein Sequence:

**MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH**  
**LYERDEGDKWRNKKFELGLEFPNLPYYIDGD**  
**VKLTQSMAI IRYIADKHNLGGCCKERA EISM**  
**LEGAVLD IRYGVSRIAYSKDFETLKVDFL**  
**SKLPEMLKMFEDRLCHKTYLNGDHVTHPD**  
**FMLYDALDVVLYMDPMCLDAFPKLVCFK**  
**KRIEAIPOIDKYLKSSKYIAWPLQGWQATFG**  
**GGDHPPKSDLEVLFGPLGSMMAAEEPQQQKQE**  
PLGSDSEGVNCLAYDEA IMAQQDRIQQEI  
AVQNP LVSERLELSVLYKEYAEDDNIY  
QQIKKDLHKKYSYIRKTRPDGNCFYRAFGE  
SHLEALLDDSKELQRFKAVSAKSKEDLVSQG  
FTEFTIEDFHNTFMDLIEQVEKQTS  
VADLLASFNDQSTSDYLVVYLRLLLTS  
GYLQRESKFFEHFIEGGRTVKEFCQQEVEPM  
CKESDHIHIIALAQALSVS IQVEYMDRGE  
GTTNPHIFPEGSEPKVYLLYRPGHYDILYK

Tag (**bold text**): N-terminal GST  
Protease cleavage site: PreScission™ (LEVLFGP) (LEVLFGP)  
OTUB1 (regular text): Start **bold italics** (amino acid residues 1-271)  
Accession number: NP\_060140



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