OTU1 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: OTU domain-containing protein 2 (OTUD2), YOD1, DUBA-8, HIV-1-induced protease 7 (HIN7)

Cat. No. **64-0036-050** Quantity: 50 µg
Lot. No. **30109** Storage: -70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

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. OTU1 is a cysteine protease and is 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 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).

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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: ~65 kDa

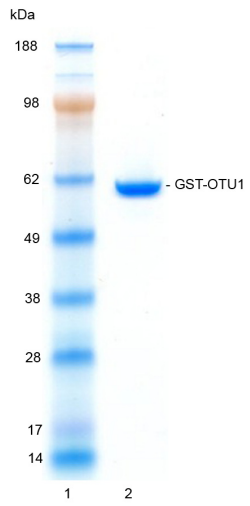
Purity: >98% by InstantBlue™ SDS-PAGE

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-OTU1



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-OTU1 was validated by determining the increase in fluorescence measured as a result of the enzyme catalysed cleavage of the fluorogenic substrate Ubiquitin-Rhodamine 110-Glycine generating Ubiquitin and Rhodamine 110-Glycine. Incubation of the substrate in the presence or absence of GST-OTU1 was compared confirming the deubiquitylating activity of GST-OTU1.

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

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). OTU1 is a constituent of a multi-protein complex with p97 as its nucleus, suggesting a functional link to a pathway responsible for the dislocation of misfolded proteins from the endoplasmic reticulum (Ernst *et al.*, 2009). p97 is an AAA ATPase that plays a central role in the ERAD pathway by chaperoning proteins to the proteasome for destruction (Messick *et al.*, 2008). In the literature, it has been shown that OTU1 binds polyubiquitin chains more tightly than monoubiquitin and preferentially hydrolyzes longer polyubiquitin chains with Lys48 linkages, having little or no activity on Lys63- and Lys29-linked chains (Messick *et al.*, 2008).

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.

Ernst R, Mueller B, Ploegh HL and Schlieker C (2009) The otubain YOD1 is a deubiquitinating enzyme that associates with p97 to facilitate protein dislocation from the ER. *Molecular Cell* **36**, 28-38.

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, et al. (2012) An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. *Nature Structural & Molecular Biology* **19**, 62-71.

Messick TE, Russell NS, Iwata AJ, Sarachan KL, Shiekhattar R, Shanks JR, et al. (2008) Structural basis for ubiquitin recognition by the Otu1 ovarian tumor domain protein. *J Biol Chem* **283**, 11038-11049.

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

Physical Characteristics

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Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVLFQGPLGS***M***FGPAK
GRHFGVHPAPGFPGGVSQQAAGTKAG
PAGAWPVGSRTDTMWRLRCKAKDGTHV
LQGLSSRTRVRELQGQIAAITGIAPGGQRIL
VGYPPECLDLSNGDTILEDLPIQSGDMLI
IEEDQTRPRSSPAFTKRGASSYVRETLPVL
TRTVVPADNSCLFTSVYYVVEGGVLNPACA
PEMRRLIAQIVASDPDFYSEAILGKTNQEYCD
WIKRDDTWGGAIEISILSKFYQCEICVVDTQT
VRIDRFGEDAGYTKRVLLIYDGIHYDPLQRN
FPDPDTPPLTIFSSNDDIVLVQALELADEAR
RRRQFTDVNRFTLRCMVCQKGLTGQAEAREHA
KETGHTNFGEV

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
OTU1 (regular text): Start ***bold italics*** (amino acid residues 1-348)
Accession number: NP_061036

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