OTUD5 (p177S) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: Deubiquitinating enzyme A, DUBA

Cat. No.	**64-0043-050**	Quantity:	50 µg
Lot. No.	**30148**	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. OTUD5 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 Kayagaki *et al.* (2007). 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 least 20 different UBD families have been described, and knowledge of linkage-specific UBDs have provided the means to understand the roles of

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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: ~87.7 kDa

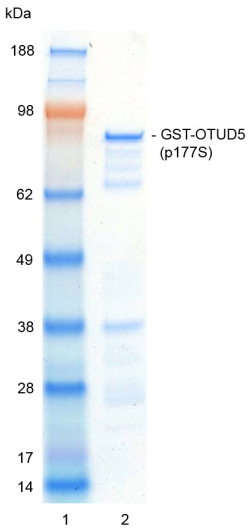
Purity: >51% by InstantBlue™ SDS-PAGE

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-OTUD5 (p177S)



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-OTUD5 (p177S) 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 GST-OTUD5 (p177S) was compared confirming the deubiquitylating activity of GST-OTUD5 (p177S).

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Lot-specific COA version tracker: v1.0.1

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different ubiquitin linkages in cells (Licchesi *et al.,* 2012). OTUD5 has been shown to selectively cleave K63–linked polyubiquitin chains on tumour necrosis factor receptor–associated factor 3 (TRAF3); an E3 ubiquitin ligase that preferentially assembles K63–linked polyubiquitin chains. Removal of these K63 polyubiquitin chains from TRAF3 results in its dissociation from the downstream signalling complex containing TANK binding kinase 1 (TBK1) (Kayagaki *et al.,* 2007). Phosphorylation of OTUD5 at a single residue, Ser177, is both necessary and sufficient to activate the enzyme. A network of interactions involving the phosphate and the C-terminal tail of ubiquitin cause OTUD5 to fold around its substrate, revealing why phosphorylation is essential for deubiquitylase activity. Phosphoactivation of OTUD5 represents an unprecedented mode of protease regulation and a clear link between two major cellular signal transduction systems: phosphorylation and ubiquitin modification (Huang *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.

Huang OW, Ma X, Yin J, Flinders J, Maurer T, Kayagaki N, *et al.* (2012) Phosphorylation-dependent activity of the deubiquitinase DUBA. *Nature Structural & Molecular Biology* **19**, 171-175.

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, 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.

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:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLEVLFQG
PLGSGF***M***TILPKKKPPPPDADPANEPPPPGP
MPPAPRRGGGVGVGGGGTGVGGGDRDRDSGV
VGARPRASPPPQGPLPGPPGALHRWALAVP
PGAVAGPRPQQASPPPCGGPGGPGGGPGDAL
GAAAAGVGAAGVVVGVGGAVGVGGCCSGPGHS
KRRRQAPGVGAVGGGSPEREEVGAGYNSEDE
YEAAAARIEAMDPATVEQQEHWFEKALRD
KKGFIIKQMKEDGACLFRAVADQVYGDQD
MHEVVRKHCMDYLMKNADYFSNYVTED
FTTYINRKRKNNCHGNHIEMQAMAEMYN
RPVEVYQYSTGTSAVEPINTFHGIHQNEDE
PIRVSYHRNIHYNSVVNPNKATIGVGLGLPS
FKPGFAEQSLMKNAIKTSEESWIEQQMLED
KKRATDWEATNEAIEEQVARESYLQWL
RDQEKQARQVRGPSQPRKASATCSSATAAASS
GLEEWTSRSPRQRSSASSPEHPELHAELGMK
PPSPGTVLALAKPPSPCAPGTSSQFSAGAD
RATSPLVSLYPALECRALIQQMSPSAFGLND
WDDDEILASVLAVSQQEYLDSMKKNKVHRDPP
PDKS

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
OTUD5 (regular text): Start ***bold italics*** (amino acid residues 1-571)
Accession number: AAH09917

Activation of OTUD5 by *in vitro* phosphorylation with Casein Kinase 2 alpha (CK2α)
OTUD5 was phosphorylated and maximally activated by incubation with 2.5% (by mass) of CK2α for 2 hours at 30˚C. The reaction was then buffer-exchanged into Enzyme Storage Buffer (50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol).
NB: The CK2α is still present in the preparation.

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