OTUD3 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: OTU domain-containing protein 3, KIAA0459

Cat. No.	**64-0035-050**	Quantity:	50 µg
Lot. No.	**30103**	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. OTUD3 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 Seki *et al.* (1999). 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 addi-

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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: ~72 kDa

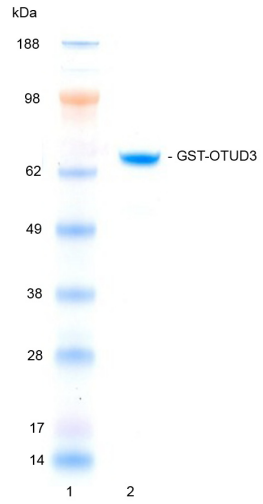
Purity: >98% by InstantBlue™ SDS-PAGE

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-OTUD3



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-OTUD3 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-OTUD3 was compared confirming the deubiquitylating activity of GST-OTUD3.

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Lot-specific COA version tracker: v1.0.0

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Background

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tional 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.

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.

Seki N, Hattori A, Hayashi A, Kozuma S, Sasaki M, Suzuki Y, *et al.* (1999) Cloning and expression profile of mouse and human genes, Rnf11/RNF11, encoding a novel RING-H2 finger protein. *Biochim Biophys Acta* **1489**, 421-427.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGS***M***SRKQAAKSR**
PGSGSRKAEAERKRDERAARRALAKERRN
RPESGGGGGCEEEFVSFANQLQALGLKL
REVPGDGNCLFRALGDQLEGHSRNHLKHRQET
VDYMIKQREDFEPFVEDDIPFEKHVASLAK
PGTFAGNDAIVAFARNHQLNVVIHQLNAPL
WQIRGTEKSSVRELHIAYRYGEHYDSVR
RINDNSEAPAHLQTDFQMLHQDESNKREKIK
TKGMDSEDDLRDEVEDAVQKVCNATGCSDF
NLIVQNLEAENYNIESAIIAVLRMNQGKRN
NAEENLEPSGRVLKQCGPLWEEGGSGARIFGN
QGLNEGRTENNKAQASPSEENKANKNQLAKVT
NKQRREQQWMEKKKRQEERHRHKALESRG
SHRDNNRSEAEANTQVTLVKTFAALNI

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
OTUD3 (regular text): Start ***bold italics*** (amino acid residues 1-629)
Accession number: NP_056022

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