OTULIN (human; full length), pAb

Alternate Names: FAM105B

Cat. No.	**68-0017-100**	Quantity:	100 µg
Lot. No.	**30254**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

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. OTULIN 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 Ota *et al.* (2004). 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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human OTULIN (residues 1-352)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects OTULIN at ~40 kDa

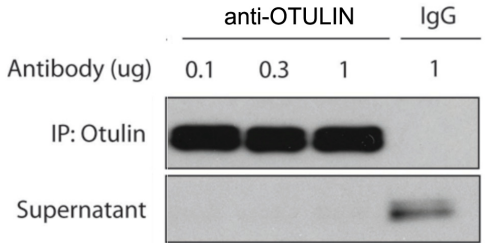
Reactivity: human; other species not tested

Stability/Storage: 12 months at -20˚C; aliquot as required

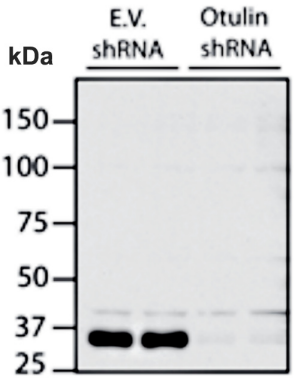
Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.5 µg/ml

Immunoprecipitation:
Use 0.3 µg/mg of cell extract



Immunoprecipitation Assay:
OTULIN was immunoprecipitated from unstimulated IL-1R HEK293 total cell extracts (0.5 mg) using various amounts of anti-OTULIN antibody (Cat# 68-0017-100) or pre-immune serum (IgG). OTULIN was subsequently detected by Western Blot using a commercially available anti-OTULIN antibody.



Western Blotting Analysis:
HEK293 IL-1R cells expressing shRNA for OTULIN or an empty vector (E.V.) were lysed and 20 µg of cell extract protein denatured in SDS and subjected to SDS-PAGE on 8% gels. Western Blotting was carried out with 0.5 µg/ml anti-OTULIN antibody (Cat# 68-0017-100).

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Lot-specific COA version tracker: v1.0.0

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Background

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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 has provided the means to understand the roles of different ubiquitin linkages in cells (Licchesi *et al.*, 2012). OTULIN (OTU DUB with linear linkage specificity) is the latest OTU to be discovered. It has been proven to specifically cleave linear ubiquitin and antagonize the E3 ligase LUBAC (linear ubiquitin chain assembly complex), thereby regulating NF-κB signalling (Wiener and Wolberger, 2013). It has been shown that the overexpression of OTULIN prevents tumour necrosis factor a (TNFα)-induced NEMO (NFκB Essential Modifier) association with ubiquitylated RIPK1, suggesting that OTULIN regulates linear poly-ubiquitin signalling (Fiil *et al.*, 2013; Keusekotten*et al.*, 2013).

Antibody Production:
Anti-OTULIN (human) polyclonal antibody was raised in sheep against OTULIN (residues 1-352 of human OTULIN). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-OTULIN pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-OTULIN (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General 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. *Nat Struct Molec Biol* **19**, 62-71.

Ota T, Suzuki Y, Nishikawa T, Otsuki T, Sugiyama T, Irie R *et al.* (2004) Complete sequencing and characterization of 21,243 full-length human cDNAs. *Nature Genetics* **36**, 40-45.

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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Lot-specific COA version tracker: v1.0.0