OTUB1 (human; full length), pAb

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

Cat. No.	**68-0016-100**	Quantity:	100 µg
Lot. No.	**30253**	Storage:	-20˚C

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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. 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 knowl-

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human OTUB1 (residues 1-271)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects OTUB1 at ~31 kDa

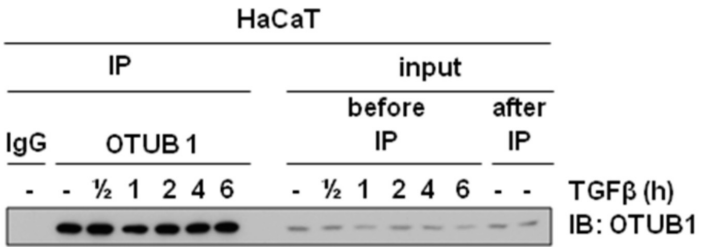
Reactivity: human; other species not tested

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

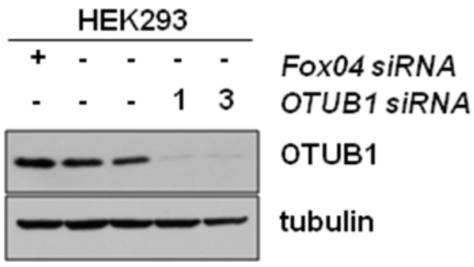
Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.1 µg/ml

Immunoprecipitation:
Use 2 µg/mg of cell extract



Immunoprecipitation Assay:
OTUB1 was immunoprecipitated from TGFβ stimulated HaCaT total cell extracts (1 mg) using 2 µg anti-OTUB1 antibody (Cat# 68-0016-100) or pre-immune serum (IgG). OTUB1 was subsequently detected by Western Blot using the same anti-OTUB1 antibody.



Western Blotting Analysis:
HEK293 cells were transfected for 48 h with 2 different siRNAs against OTUB1 (1 and 3). The cells were then lysed and the lysates denatured in SDS and subjected to SDS-PAGE on 8% gels. Western Blotting was carried out with 0.1 µg/ml anti-OTUB1 (Cat# 68-0016-100) or with an anti-tubulin antibody.

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Dundee, Scotland, UK

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Email: sales.support@ubiquigent.com

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International: +44 (0) 1382 381147 (9AM-5PM UTC)
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Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

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edge of linkage-specific UBDs has provided the means to understand the roles of different ubiquitin linkages in 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 are 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) signalling, indicating that pharmacological targeting of the OTUB1-UBE2N interaction might enhance the DNA damage response (Blackford and Stewart, 2011; Nakada *et al.*, 2010).

Antibody Production:
Anti-OTUB1 (human) polyclonal antibody was raised in sheep against OTUB1 (residues 1-271 of human OTUB1). 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-OTUB1 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-OTUB1 (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.

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, 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.

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. EMBO J **31**, 576-592.

Application Reference:

Herhaus L, Al-Salihi M, Macartney T, Weidlich S, Sapkota GP (2013) OTUB1 enhances TGFβ signalling by inhibiting the ubiquitylation and degradation of active SMAD2/3. *Nat Commun* **4**, 2519.

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