USP15 (human; full length), pAb

Alternate Names: Unph2, Unph4

Cat. No.	**68-0029-100**	Quantity:	100 µg
Lot. No.	**30266**	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. Ubiquitin carboxyl-terminal hydrolase 15 is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Baker *et al.* (1999). USP15 functions in COP9 signalosome-mediated regulation of protein degradation and cellular signalling through catalysing the ubiquitin deconjugation reaction of a discrete number of substrates (Harper *et al.*, 2011). USP15 plays a role in the downregulation of the NF-κB

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human USP15 (residues 1-952)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects USP15 at ~112 kDa

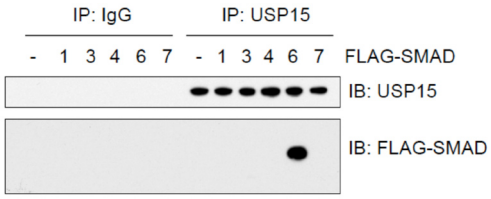
Reactivity: human; other species not tested

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

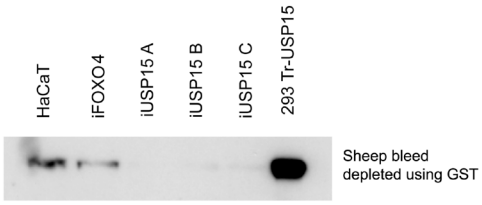
Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.1 µg/ml

Immunoprecipitation:
Use 2 µg/mg of cell extract



Immunoprecipitation Assay:
HEK293 cells were transfected with a control vector (-) or FLAG-SMADs (SMAD1, 3, 4, 6 or 7) as indicated. USP15 was immunoprecipitated from each HEK293 total cell extract (1 mg) using 2 µg of anti-USP15 antibody (Cat# 68-0029-100). USP15 was subsequently detected by Western Blot using a commercially sourced anti-USP15 antibody or with an anti-FLAG-SMAD antibody.



Western Blotting Analysis:
HaCaT cells were transfected with siRNA against USP15 (iUSP15A-C) or siRNA targeting FOXO4 (iFOXO4) as a control. HEK293 cells transfected with USP15 (293 Tr-USP15) were used as a Western Blotting positive control. 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-USP15 antibody (Cat# 68-0029-100) on 20 µg total cell extract.

UBIQUIGENT™
www.ubiquigent.com
Dundee, Scotland, UK

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Email: tech.support@ubiquigent.com

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Lot-specific COA version tracker: v1.0.0

USP15 (human; full length), pAb

Alternate Names: Unph2, Unph4

Cat. No.	**68-0029-100**	Quantity:	100 µg
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CERTIFICATE OF ANALYSIS

Background

Continued from page 1

pathway through deubiquitylating the NF-κB inhibitor IκBα and also regulates human papillomavirus type 16 E6 oncoprotein stability. Among USPs, USP15 is most closely related to USP4 which has recently been implicated in mRNA splicing and more distantly to USP11 which has been linked to DNA damage repair pathways (Harper *et al.*, 2011). It has recently been demonstrated that USP15 plays an essential role in the stabilisation and activity of caspase-3 during Paclitaxel-induced apoptosis. It has therefore been proposed that USP15 may be a candidate diagnostic marker and therapeutic target for Paclitaxel-resistant cancers (Xu *et al.*, 2009).

Antibody Production:
Anti-USP15 (human) polyclonal antibody was raised in sheep against USP15 (residues 1-952 of human USP15). 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-USP15 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-USP15 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Baker RT, Wang XW, Woollatt E, White JA, Sutherland GR (1999) Identification, functional characterization, and chromosomal localization of USP15, a novel human ubiquitin-specific protease related to the UNP oncoprotein, and a systematic nomenclature for human ubiquitin-specific proteases. *Genomics* **59**, 264-274.

Harper S, Besong TM, Emsley J, Scott DJ, Dreveny I (2011) Structure of the USP15 N-terminal domains: a beta-hairpin mediates close association between the DUSP and UBL domains. *Biochemistry* **50**, 7995-8004.

Komander D, Clague MJ, Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-563.

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

Xu M, Takanashi M, Oikawa K, Tanaka M, Nishi H, Isaka K, Kudo M, Kuroda M (2009) USP15 plays an essential role for caspase-3 activation during Paclitaxel-induced apoptosis. *Biochem Biophys Res Commun* **388**, 366-371.

Application Reference:

Al-Salihli MA, Herhaus L, Macartney T and Sapkota GP (2012) USP11 augments TGFbeta signalling by deubiquitylating ALK5. *Open Biology* **2**, 120063.

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Lot-specific COA version tracker: v1.0.0