

## Product datasheet

# Anti-OTUB1 antibody [EPR13028(B)] ab175200

**KO VALIDATED** Recombinant RabMAB

★★★★★ [3 Abreviews](#) [10 References](#) [7 Images](#)

### Overview

<b>Product name</b>	Anti-OTUB1 antibody [EPR13028(B)]
<b>Description</b>	Rabbit monoclonal [EPR13028(B)] to OTUB1
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> WB, IP <b>Unsuitable for:</b> Flow Cyt, ICC/IF or IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide within Human OTUB1 aa 200 to the C-terminus (Cysteine residue). The exact sequence is proprietary. Database link: <a href="#">Q96FW1</a>
<b>Positive control</b>	WB: Wild-type HAP1, HeLa, MCF7, HepG2, HEK-293T, and HEK-293 cell lysates. Rat and mouse heart tissue lysates. IP: HeLa cell lysate.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAB<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAB<sup>®</sup> patents</a>.</p> <p><b>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</b></p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.

<b>Storage buffer</b>	Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR13028(B)
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab175200 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (2)	1/1000 - 1/10000. Predicted molecular weight: 31 kDa.
IP	★★★★★ (1)	1/10 - 1/100.

**Application notes** Is unsuitable for Flow Cyt, ICC/IF or IHC-P.

## Target

**Function** Hydrolase that can remove conjugated ubiquitin from proteins and plays an important regulatory role at the level of protein turnover by preventing degradation. Regulator of T-cell anergy, a phenomenon that occurs when T-cells are rendered unresponsive to antigen rechallenge and no longer respond to their cognate antigen. Acts via its interaction with RNF128/GRAIL, a crucial inductor of CD4 T-cell anergy. Isoform 1 destabilizes RNF128, leading to prevent anergy. In contrast, isoform 2 stabilizes RNF128 and promotes anergy. Surprisingly, it regulates RNF128-mediated ubiquitination, but does not deubiquitinate polyubiquitinated RNF128. Deubiquitinates estrogen receptor alpha (ESR1). Mediates deubiquitination of 'Lys-48'-linked polyubiquitin chains, but not 'Lys-63'-linked polyubiquitin chains. Not able to cleave di-ubiquitin. Also capable of removing NEDD8 from NEDD8 conjugates, but with a much lower preference compared to 'Lys-48'-linked ubiquitin.

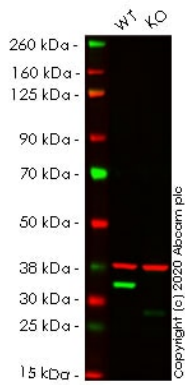
**Tissue specificity** Isoform 1 is ubiquitous. Isoform 2 is expressed only in lymphoid tissues such as tonsils, lymph nodes and spleen, as well as peripheral blood mononuclear cells.

**Sequence similarities** Belongs to the peptidase C65 family.  
Contains 1 OTU domain.

**Domain** In addition to ubiquitin-binding at the Cys-91 active site, a proximal ubiquitin-binding site is also present at Cys-23. Occupancy of the active site is needed to enable tight binding to the second site. Distinct binding sites for the ubiquitins may allow to discriminate among different isopeptide linkages (i.e. 'Lys-48-', 'Lys-63'-linked polyubiquitin) in polyubiquitin substrates and achieve linkage-specific deubiquitination.

**Cellular localization** Cytoplasm.

## Images



Western blot - Anti-OTUB1 antibody [EPR13028(B)]  
(ab175200)

**All lanes** : Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution

**Lane 1** : Wild-type HEK-293T cell lysate

**Lane 2** : OTUB1 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

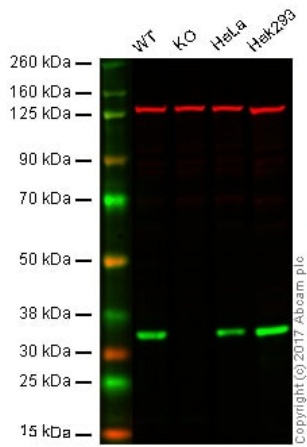
Performed under reducing conditions.

**Predicted band size:** 31 kDa

**Observed band size:** 31 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab175200 observed at 31 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab175200 Anti-OTUB1 antibody [EPR13028(B)] was shown to specifically react with OTUB1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266551** (knockout cell lysate **ab257569**) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. ab175200 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 Dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

**All lanes :** Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution

**Lane 1 :** Wild-type HAP1 whole cell lysate

**Lane 2 :** OTUB1 knockout HAP1 whole cell lysate

**Lane 3 :** HeLa whole cell lysate

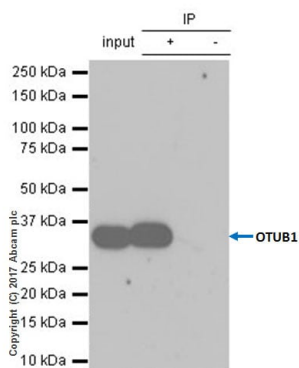
**Lane 4 :** HEK-293 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 31 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab175200 observed at 35 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab175200 was shown to specifically react with OTUB1 in wild type cells as signal was lost in OTUB1 knockout cells. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. ab175200 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

ab175200 (purified) at 1:50 dilution (2ug) immunoprecipitating OTUB1 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

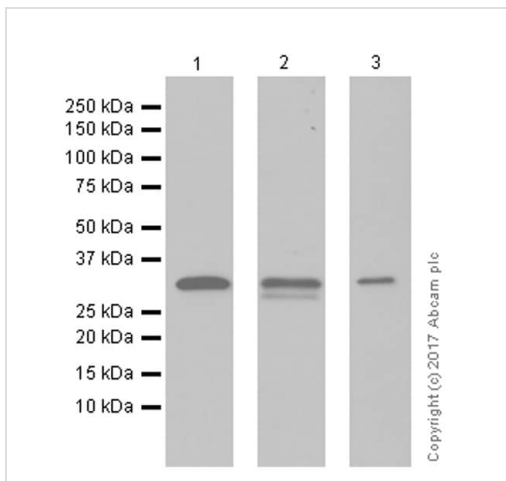
**Lane 1 (input):** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

**Lane 2 (+):** ab175200 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

**Lane 3 (-):** Rabbit monoclonal IgG (**ab172730**) instead of ab175200 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFD/DM/TBST.



Western blot - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

**All lanes** : Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution (purified)

**Lane 1** : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

**Lane 2** : Mouse heart whole tissue lysates

**Lane 3** : Rat heart whole tissue lysates

Lysates/proteins at 15 µg per lane.

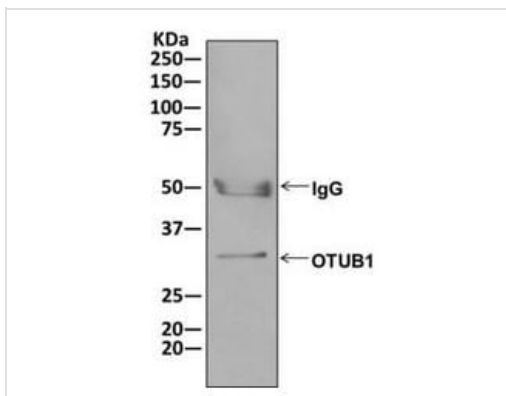
**Secondary**

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 31 kDa

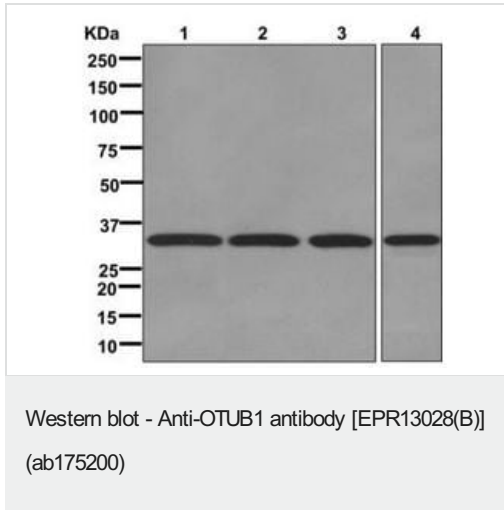
**Observed band size:** 31 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Immunoprecipitation - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

Western blot analysis on immunoprecipitation pellet from MCF7 cell lysate labeling OTUB1 with unpurified ab175200 at 1/10 dilution.



**All lanes :** Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution (unpurified)

**Lane 1 :** HeLa cell lysate

**Lane 2 :** HepG2 cell lysate

**Lane 3 :** 293T cell lysate

**Lane 4 :** MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

**Predicted band size:** 31 kDa

Why choose a recombinant antibody?

- Research with confidence**  
Consistent and reproducible results
- Long-term and scalable supply**  
Recombinant technology
- Success from the first experiment**  
Confirmed specificity
- Ethical standards compliant**  
Animal-free production

Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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