abcam

Product datasheet

Anti-OTUB1 antibody [EPR13028(B)] - BSA and Azide free ab232581

KO VALIDATED Recombinant RabMAb

5 Images

Overview

Product name	Anti-OTUB1 antibody [EPR13028(B)] - BSA and Azide free
Description	Rabbit monoclonal [EPR13028(B)] to OTUB1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP **Unsuitable for:** Flow Cyt,ICC/IF or IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1, HeLa, MCF7, HepG2, HEK-293T, and HEK-293 cell lysates. Rat and mouse heart tissue lysates. IP: HeLa cell lysate.
General notes	ab232581 is the carrier-free version of **ab175200**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR13028(B)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232581 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		Use at an assay dependent concentration. Predicted molecular weight: 31 kDa.
IP		Use at an assay dependent concentration.

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

Target

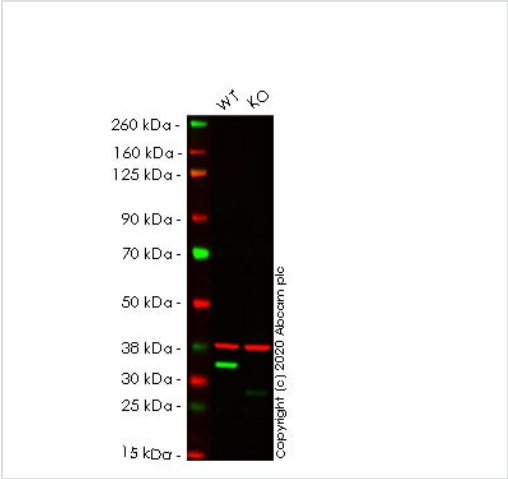
Function Hydrolase that can remove conjugated ubiquitin from proteins and plays an important regulatory role at the level of protein turnover by preventing degradation. Regulator of T-cell anergy, a phenomenon that occurs when T-cells are rendered unresponsive to antigen rechallenge and no longer respond 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 nuch 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)] - BSA and Azide free (ab232581)

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.

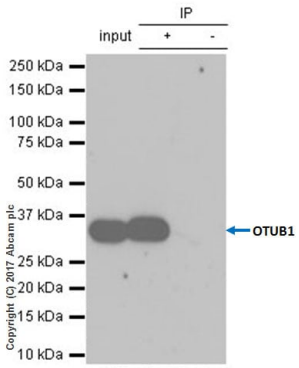
Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 130 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab175200**).

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°CC 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.



Immunoprecipitation - Anti-OTUB1 antibody [EPR13028(B)] - BSA and Azide free (ab232581)

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

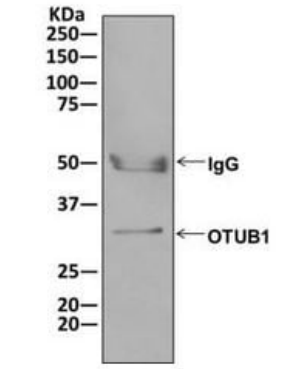
Lane 2 (+): **ab175200** & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

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% NFDM/TBST.

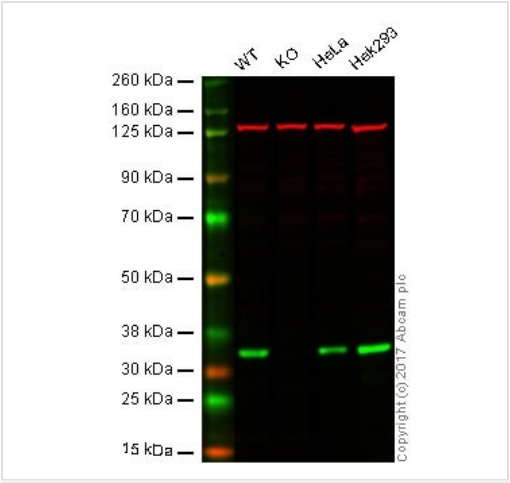
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab175200**).



Immunoprecipitation - Anti-OTUB1 antibody [EPR13028(B)] - BSA and Azide free (ab232581)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab175200**).



Western blot - Anti-OTUB1 antibody [EPR13028(B)] - BSA and Azide free (ab232581)

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 1/1,000 dilution and 1/20,000 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 1/20,000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab175200**).

Anti-OTUB1 antibody [EPR13028(B)] - BSA and Azide free (ab232581)

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