abcam

Product datasheet

Anti-OTUB1 antibody [EPR24917-75] ab270959

KO VALIDATED Recombinant RabMAb

2 References 9 Images

Overview

Product name	Anti-OTUB1 antibody [EPR24917-75]
Description	Rabbit monoclonal [EPR24917-75] to OTUB1
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, WB, Flow Cyt (Intra) **Unsuitable for:** 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: WT HEK293T, HeLa, MCF7, U-2 OS, Mouse liver, Mouse brain, Rat liver, Rat brain, C6, RAW264.7, PC-12, NIH/3T3 lysates. ICC: HeLa, WT HEK293T cells. Flow Cyt Intra: Wild-type HEK293T, HeLa cells. IP: HeLa cells.
General notes	**Please note**: IP, FC and ICC are valid for Human samples only 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR24917-75
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab270959 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
IP		1/30.
ICC/IF		1/250.
WB		1/1000. Predicted molecular weight: 31 kDa.
Flow Cyt (Intra)		1/500.

Application notes Is unsuitable for 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.

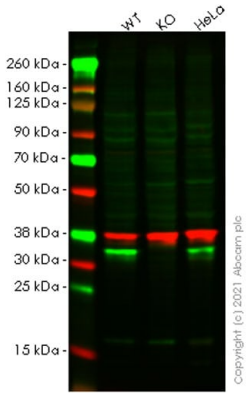
Tissue specificity Isoform 1 is ubiquitous. Isoform 2 is expressed only in lymphoid tissues such as tonsils, lymph nodes and spleen, as well as peripheral blood mononuclear cells.

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 [EPR24917-75] (ab270959)

All lanes : Anti-OTUB1 antibody [EPR24917-75] (ab270959) at 1/1000 dilution

Lane 1 : Wild-type HEK293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : OTUB1 knockout HEK293T (**ab266551**) whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

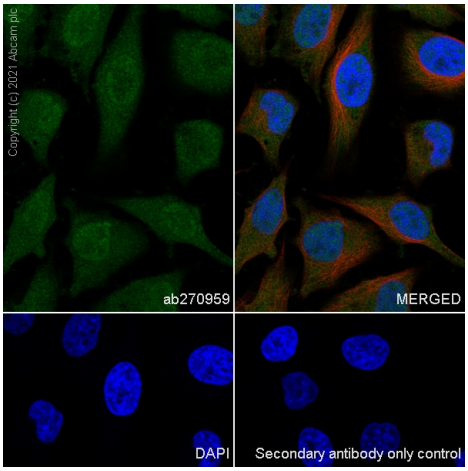
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

Predicted band size: 31 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Lanes 1-3: Merged signal (red and green). Green -ab270959 observed at 31kDa. Red - loading control **ab8245** observed at 36 kDa. ab270959 Anti-OTUB1 antibody [EPR24917-75] was shown to specifically react with OTUB1 in wild-type HEK293T 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.

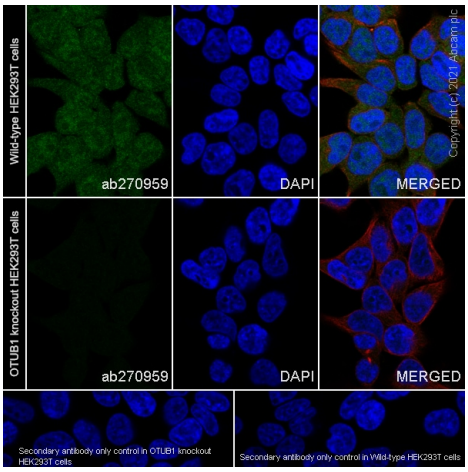
ab270959 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling OTUB1 with ab270959 at 1/250 (2.204 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing nuclear and cytoplasmic staining in HeLa cell line is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

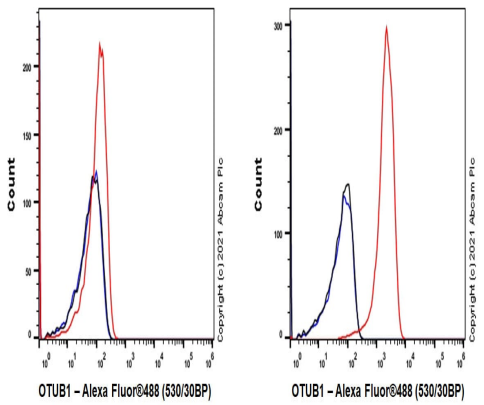
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

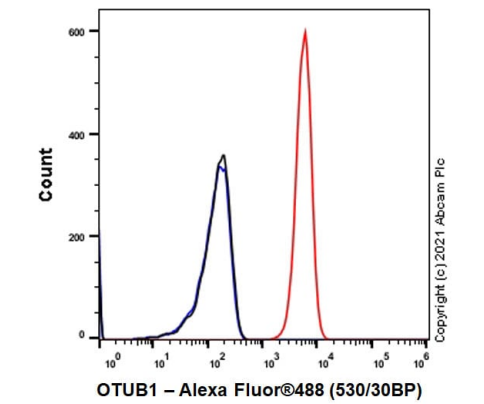
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized OTUB1 KO HEK293T (**ab266551**) cells labelling OTUB1 with ab270959 at 1/250 (2.204 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing no staining in OTUB1 KO HEK293T cell line and nuclear and cytoplasmic staining in Parental HEK293T is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



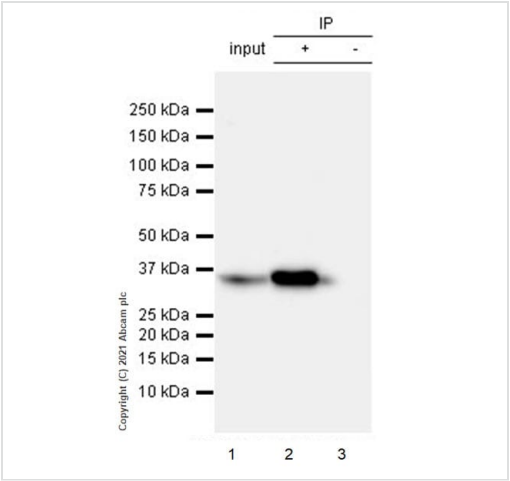
Flow Cytometry (Intracellular) - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Wild-type HEK293T (human embryonic kidney epithelial cell, Right)/ OTUB1 KO HEK293T (Left) cells labelling OTUB1 with ab270959 at 1/500 dilution (0.1ug)/ (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody. Positive staining on 293T cells (**ab255449**), while no staining on OTUB1 knockout HEK-293T cells (**ab266551**).



Flow Cytometry (Intracellular) - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling OTUB1 with ab270959 at 1/500 dilution (0.1ug)/ (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

OTUB1 was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate 10ug with ab270959 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab270959 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

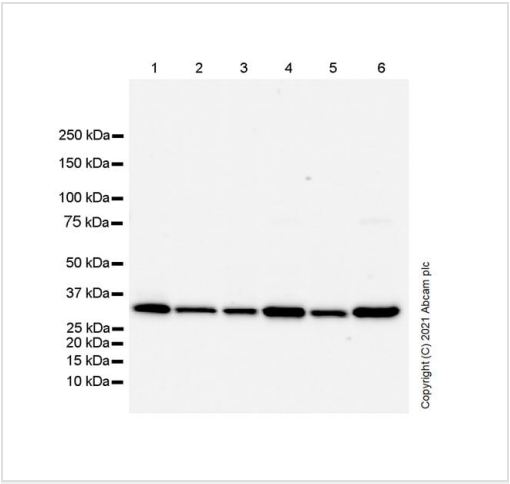
Lane 1: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate 10ug

Lane 2: ab270959 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab270959 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 7 seconds



Western blot - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

All lanes : Anti-OTUB1 antibody [EPR24917-75] (ab270959) at 1/1000 dilution

Lane 1 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : U-2 OS(human bone osteosarcoma epithelial cell), whole cell lysate
Lane 3 : Mouse liver tissue lysate
Lane 4 : Mouse brain tissue lysate
Lane 5 : Rat liver tissue lysate
Lane 6 : Rat brain tissue lysate

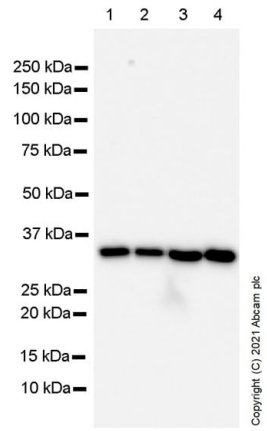
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 31 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 26 seconds



Western blot - Anti-OTUB1 antibody [EPR24917-75] (ab270959)

All lanes : Anti-OTUB1 antibody [EPR24917-75] (ab270959) at 1/1000 dilution

Lane 1 : C6 (rat glial tumor glial cell), whole cell lysate
Lane 2 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate
Lane 4 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 31 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 15 seconds

Anti-OTUB1 antibody [EPR24917-75] (ab270959)

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