abcam

ab283705

Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR24917-75
Purification technique	Affinity purification Protein A
Concentration	1.011 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information OTUB1

Function

Hydrolase that can specifically remove 'Lys-48'-linked conjugated ubiquitin from proteins and plays an important regulatory role at the level of protein turnover by preventing degradation (PubMed:12401499, PubMed:12704427, PubMed:14661020, PubMed:23827681). 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 (PubMed:14661020). Acts via its interaction with RNF128/GRAIL, a crucial inductor of CD4 T-cell anergy (PubMed:14661020). Isoform 1 destabilizes RNF128, leading to prevent anergy (PubMed:14661020). In contrast, isoform 2 stabilizes RNF128 and promotes anergy (PubMed:14661020). Surprisingly, it regulates RNF128-mediated ubiquitination, but does not deubiquitinate polyubiquitinated RNF128 (PubMed:14661020). Deubiquitinates estrogen receptor alpha (ESR1) (PubMed:19383985). Mediates deubiquitination of 'Lys-48'-linked polyubiquitin chains, but not 'Lys-63'-linked polyubiquitin chains (PubMed:18954305, PubMed:19211026, PubMed:23827681). Not able to cleave di-ubiquitin (PubMed:18954305, PubMed:23827681). Also capable of removing NEDD8 from NEDD8 conjugates, but with a much lower preference compared to 'Lys-48'-linked ubiquitin (PubMed:18954305, PubMed:23827681). Plays a key non-catalytic role in DNA repair regulation by inhibiting activity of RNF168, an E3 ubiquitin-protein ligase that promotes accumulation of 'Lys-63'-linked histone H2A and H2AX at DNA damage sites (PubMed:20725033, PubMed:22325355). Inhibits RNF168 independently of ubiquitin thioesterase activity by binding and inhibiting UBE2N/UBC13, the E2 partner of RNF168, thereby limiting spreading of 'Lys-63'-linked histone H2A and H2AX marks (PubMed:20725033, PubMed:22325355). Inhibition occurs by binding to free ubiquitin: free ubiquitin acts as an allosteric regulator that increases affinity for UBE2N/UBC13 and disrupts interaction with UBE2V1 (PubMed:20725033, PubMed:22325355). The OTUB1-UBE2N/UBC13-free ubiquitin complex adopts a configuration that mimics a cleaved 'Lys48'-linked di-ubiquitin

chain (PubMed:20725033, PubMed:22325355). Acts as a regulator of mTORC1 and mTORC2 complexes (PubMed:29382726, PubMed:35927303). When phosphorylated at Tyr-26, acts as an activator of the mTORC1 complex by mediating deubiquitination of RPTOR via a non-catalytic process: acts by binding and inhibiting the activity of the ubiquitin-conjugating enzyme E2 (UBE2D1/UBCH5A, UBE2W/UBC16 and UBE2N/UBC13), thereby preventing ubiquitination of RPTOR (PubMed:35927303). Can also act as an inhibitor of the mTORC1 and mTORC2 complexes in response to amino acids by mediating non-catalytic deubiquitination of DEPTOR (PubMed:29382726).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

Please note: IP, FC and ICC are valid for Human samples only.
ab283705 is the carrier-free version of ab270959.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The OTUB1 protein also known as OTU domain-containing ubiquitin aldehyde-binding protein 1 plays an important role in the removal of ubiquitin from proteins. This process is important for regulating protein stability and function within the cell. OTUB1 has a mass of approximately 32 kDa and is expressed ubiquitously across various tissues indicating its essential nature in cellular functions.
Biological function summary	OTUB1 functions as a deubiquitinating enzyme that cleaves ubiquitin from substrates influencing various cellular processes. It acts independently or as part of larger protein complexes. By controlling protein degradation pathways OTUB1 impacts protein turnover and cellular homeostasis. Interactions with other proteins allow OTUB1 to exert influence on different signaling pathways and cellular stress responses.
Pathways	OTUB1 holds a significant position in the regulation of the ubiquitin-proteasome system and DNA damage repair pathway. It closely interacts with proteins such as UBE2N affecting the DNA damage response. Additionally OTUB1 regulates processes through modulating pathways like NF-kB by interacting with and stabilizing specific protein factors involved.
Associated diseases and disorders	OTUB1's function connects it to various pathological states like cancer and neurodegenerative diseases. Aberrant expression or mutations in OTUB1 can lead to disruptions in cell cycle regulation and apoptosis contributing to oncogenesis. In the context of neurodegenerative diseases OTUB1 is related to tau stabilization with proteins such as HSP90 playing a role in the same regulatory mechanisms highlighting its relevance in these disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

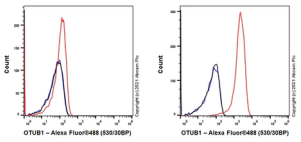
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

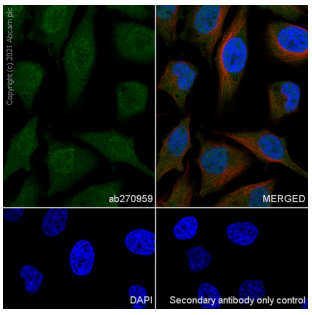
8 product images



Flow Cytometry (Intracellular) - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Flow Cytometry (Intracellular) staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
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).

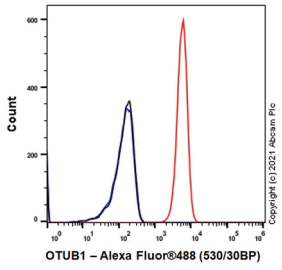


Immunocytochemistry/ Immunofluorescence - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
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.

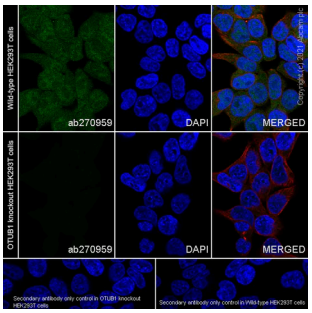


Flow Cytometry (Intracellular) - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Flow Cytometry (Intracellular) staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
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.

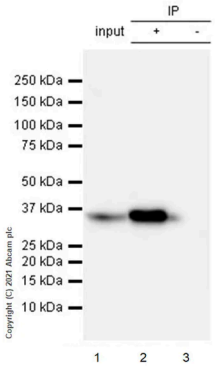


Immunocytochemistry/ Immunofluorescence - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
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.



Immunoprecipitation - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
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

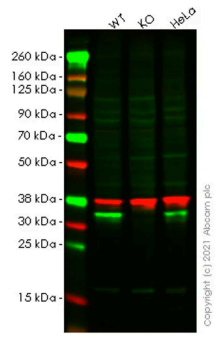
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab270959 in HeLa whole cell lysate

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

Exposure time: 7 seconds

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

Predicted band size: 31 kDa



Western blot - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Western blot staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
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.

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

Lane 1:
Wild-type HEK293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

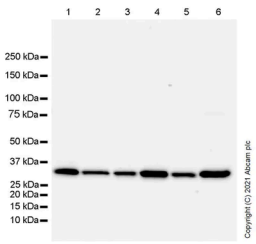
Lane 2:
OTUB1 knockout HEK293T (ab266551) whole cell lysate at 20 µg

Lane 3:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

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



Western blot - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Western blot staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 26 seconds

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

Lane 1:
MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:

U-2 OS(human bone osteosarcoma epithelial cell), whole cell lysate at 20 µg

Lane 3:
Mouse liver tissue lysate at 20 µg

Lane 4:
Mouse brain tissue lysate at 20 µg

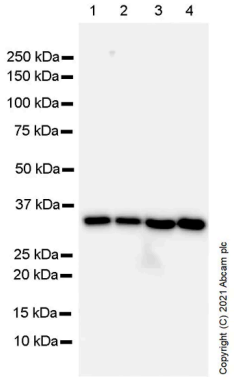
Lane 5:
Rat liver tissue lysate at 20 µg

Lane 6:
Rat brain tissue lysate at 20 µg

Secondary

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

Predicted band size: 31 kDa



Western blot - Anti-OTUB1 antibody [EPR24917-75] - BSA and Azide free (ab283705)

OTUB1 Western blot staining using rabbit Anti-OTUB1 antibody

This data was developed using ab270959, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 15 seconds

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

Lane 1:
C6 (rat glial tumor glial cell), whole cell lysate at 20 µg

Lane 2:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate at 20 µg

Lane 3:
PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate at 20 µg

Lane 4:
NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Secondary

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

Predicted band size: 31 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.