abcam

ab52472

Anti-ATG7 antibody [EP1759Y]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP1759Y
Purification technique	Affinity purification Protein A
Concentration	0.53 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30 - 1/50
Notes	-

WB

Tested

Species	Human
Dilution info	1/100000 - 1/200000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/500
Notes	**For unpurified use at 10 µg/mL.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	1/500
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information ATG7

Function	E1-like activating enzyme involved in the 2 ubiquitin-like systems required for cytoplasm to vacuole transport (Cvt) and autophagy. Activates ATG12 for its

conjugation with ATG5 as well as the ATG8 family proteins for their conjugation with phosphatidylethanolamine. Both systems are needed for the ATG8 association to Cvt vesicles and autophagosomes membranes. Required for autophagic death induced by caspase-8 inhibition. Facilitates LC3-I lipidation with phosphatidylethanolamine to form LC3-II which is found on autophagosomal membranes (PubMed:34161705). Required for mitophagy which contributes to regulate mitochondrial quantity and quality by eliminating the mitochondria to a basal level to fulfill cellular energy requirements and preventing excess ROS production. Modulates p53/TP53 activity to regulate cell cycle and survival during metabolic stress. Also plays a key role in the maintenance of axonal homeostasis, the prevention of axonal degeneration, the maintenance of hematopoietic stem cells, the formation of Paneth cell granules, as well as in adipose differentiation. Plays a role in regulating the liver clock and glucose metabolism by mediating the autophagic degradation of CRY1 (clock repressor) in a time-dependent manner (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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.

Supplementary info

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

Activity summary	ATG7 also known as Autophagy Related 7 is an essential protein involved in the autophagy process. It functions as an E1-like enzyme activating and transferring ubiquitin-like proteins such as ATG12 and LC3. The molecular weight of ATG7 is approximately 78 kDa. It is widely expressed in various tissues although expression levels can differ. One can detect ATG7 using immunoassays like ELISA or antibodies specifically targeting ATG7. Understanding its mechanical role is key to studying cellular homeostasis.
Biological function summary	ATG7 plays a significant role in autophagy a cellular degradation pathway critical for cell survival under stress. ATG7 contributes to the formation of autophagosomes by facilitating conjugation of ATG8 family proteins including LC3 to phosphatidylethanolamine. It acts within complexes that regulate cellular energy balance and stress responses ensuring cells maintain their function and integrity. Knockdown of ATG7 can impair autophagic flux highlighting its importance in maintaining cellular processes.
Pathways	ATG7 is a central player in the autophagy pathway influencing cellular metabolism and turnover. It interacts closely with ATG5 and ATG12 in this pathway to form a conjugation system essential for autophagosome elongation. Additionally ATG7 is involved in the mTOR signaling pathway which regulates nutrient sensing and cellular growth. Interaction with proteins like mTOR allows ATG7 to integrate signals from nutrient availability and stress responses finely tuning the autophagy process.
Associated diseases and disorders	ATG7 dysfunction has connections to cancer and neurodegenerative diseases like Alzheimer's. Abnormal ATG7 activity disrupts autophagic balance possibly leading to the accumulation of damaged proteins and organelles contributing to disease progression. In cancer altered ATG7 expression may influence tumor survival by affecting cellular stress responses. Proteins such as p53 involved in cell cycle regulation often show association with ATG7-related pathways indicating a complex network influencing disease states. Understanding ATG7's role in these conditions can help explore potential therapeutic strategies.

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.

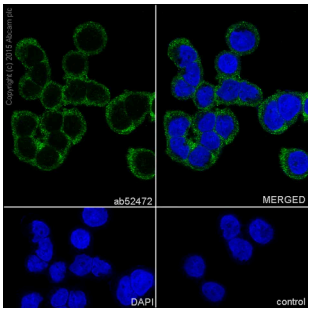
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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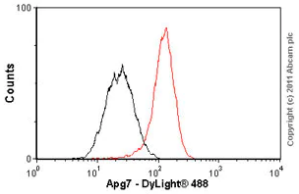
11 product images



Immunocytochemistry/ Immunofluorescence - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG7 antibody

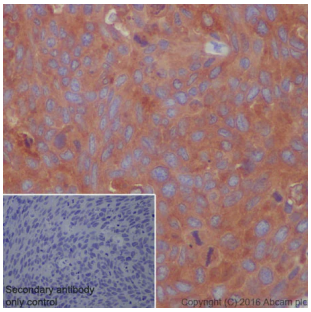
Immunocytochemistry/Immunofluorescence analysis of HT-29 (human colorectal adenocarcinoma) cells labelling ATG7 with purified ab52472 at 1/500. Cells were fixed with 100% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (ab150077) at 1/1000 dilution was used as the secondary antibody. Nuclei couterstained with DAPI (blue).
Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Flow Cytometry (Intracellular) - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Flow Cytometry (Intracellular) staining using rabbit Anti-ATG7 antibody

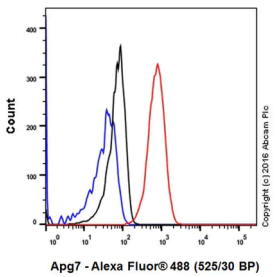
Overlay histogram showing HEK293 cells stained with unpurified ab52472 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab52472, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 100% methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ATG7 antibody

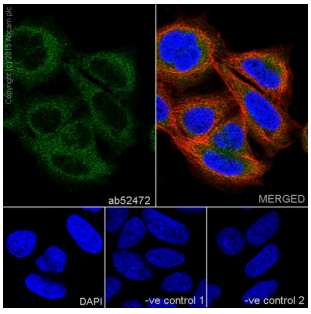
Immunohistochemical analysis of paraffin-embedded human cervical carcinoma sections labelling ATG7 with purified ab52472 at a dilution of 1/500. The secondary antibody used was ab97051, Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Flow Cytometry (Intracellular) - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Flow Cytometry (Intracellular) staining using rabbit Anti-ATG7 antibody

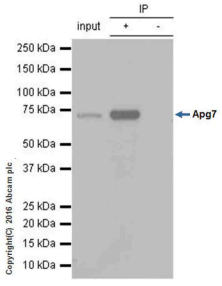
Intracellular Flow Cytometry analysis of HeLa cells labelling ATG7 (red) with purified ab52472 at dilution of 1/100. The secondary antibody used was goat anti rabbit IgG (FITC) at 1/500. Cells were fixed with 4% paraformaldehyde. Isotype control antibody was Rabbit monoclonal IgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG7 antibody

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ATG7 with purified ab52472 at 1/100. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% Triton X-100. ab150077, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with ab7291 anti-Tubulin (mouse mAb) followed by ab150120, AlexaFluor®594 goat anti-mouse secondary both at 1/1000. Nuclei were counterstained with DAPI (blue).
For negative control 1, rabbit primary antibody was used followed by anti-mouse secondary antibody (ab150120). For negative control 2, ab7291 (mouse primary antibody) was used followed by anti-rabbit secondary antibody (ab150077).



Immunoprecipitation - Anti-ATG7 antibody [EP1759Y] (ab52472)

ab52472 at 1/30 dilution immunoprecipitating ATG7 in HEK293 whole cell lysate observed at 70 KDa (lanes 1 and 2).
Lane 1 (input): HEK293 whole cell lysate 10ug

Lane 2 (+): ab52472 + HEK293 whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab52472 in HEK293 whole cell lysate

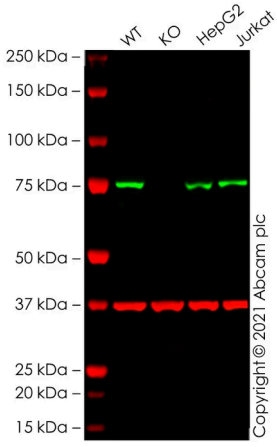
For western blotting, ab52472 was used followed by VeriBlot for IP Detection Reagent (HRP) (ab131366) for detection at a dilution of 1/10,000.

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

All lanes:

Immunoprecipitation - Anti-ATG7 antibody [EP1759Y] (ab52472)

Predicted band size: 49 kDa, 77 kDa



Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Western blot staining using rabbit Anti-ATG7 antibody

False colour image of Western blot: Anti-ATG7 antibody [EP1759Y] staining at 1/100000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab52472 was shown to bind specifically to ATG7. A band was observed at 75 kDa in wild-type HeLa cell lysates with no signal observed at this size in ATG7 knockout cell line ab283307 (knockout cell lysate ab287353). To generate this image, wild-type and ATG7 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472) at 1/100000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human ATG7 knockout HeLa cell lysate (ab287353) at 20 µg

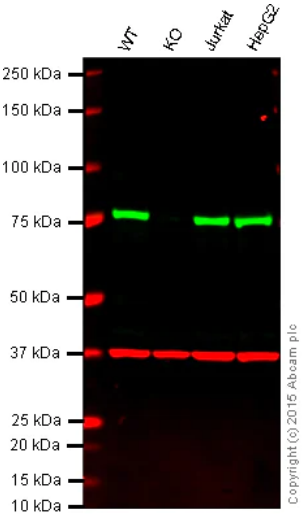
Lane 3:
HepG2 cell lysate at 20 µg

Lane 4:
Jurkat cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 77 kDa

Observed band size: 75 kDa



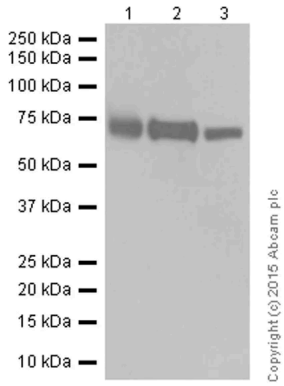
Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Western blot staining using rabbit Anti-ATG7 antibody

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: ATG7 knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab52472 observed at 77 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab52472 was shown to specifically react with ATG7 when ATG7 knockout samples were used. Wild-type and ProteinX knockout samples were subjected to SDS-PAGE. ab52472 and ab8245 (loading control to Apg7) were both diluted 1/2000 and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.

All lanes:
Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472)

Predicted band size: 77 kDa



Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Western blot staining using rabbit Anti-ATG7 antibody

Blocking and Diluting buffer 5% NFDM/TBST
All lanes:
Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472) at 1/100000 dilution

Lane 1:
HEK293 whole cell lysate at 20 µg

Lane 2:
HepG2 whole cell lysate at 20 µg

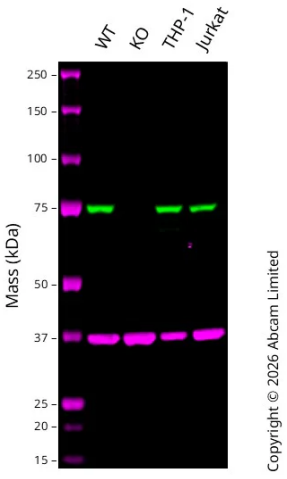
Lane 3:
Jurkat whole cell lysate at 20 µg

Secondary

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

Predicted band size: 77 kDa

Observed band size: 70 kDa



Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Western blot staining using rabbit Anti-ATG7 antibody

Western blot: Anti-ATG7 antibody [EP1759Y] ab52472 staining at 1/2000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 78 kDa in Wild-type U-87 MG cell lysates with no signal observed at this size in ATG7 knockout U-87 MG cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472) at 1/2000 dilution

Lane 1:
Wild-type U-87 MG at 20 µg

Lane 2:
ATG7 knockout U-87 MG at 20 µg

Lane 3:
THP-1 at 20 µg

Lane 4:
Jurkat at 20 µg

Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 78 kDa

Observed band size: 78 kDa

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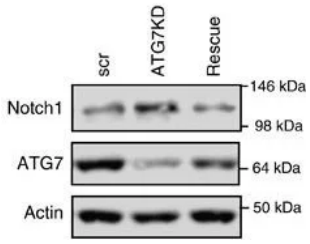


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-ATG7 antibody [EP1759Y] (ab52472)

ATG7 Western blot staining using rabbit Anti-ATG7 antibody

Western Blotting using Anti-ATG7 antibody [EP1759Y], ab52472. Publication image from Rubinsztein, D. C. et al., 2016, Nat Commun, 26837467. Legend direct from paper.

Autophagy modulates Notch signalling pathway.(a) Representative western blot showing levels of Notch1 and its downstream effectors. Autophagy was inhibited in HEK cells by Smartpool siRNA (KD) for ATG7 or ATG16L1 or activated by pcDNA/Beclin-Flag transfection. Scr=scrambled siRNA. Actin is loading control. (b) Quantification of western blots for Notch1 and effectors, relative to actin, normalized for relevant control. *P<0.05 or **P<0.01 by paired t-test. n=3 in triplicates. Error bars=s.e.m. (c) Representative western blot showing effect of ATG7 Smartpool siRNA knockdown and ATG7-wt cDNA rescue on Notch1 levels. (d) Quantification of western blots for Notch1 and ATG7, relative to actin, normalized for relevant control. *P<0.05 or **P<0.01 by paired t-test. NS denotes not significant. n=4. Error bars=s.e.m. (e) Representative western blot showing the effect of ATG16L1 Smartpool siRNA knockdown and ATG16L1 mStr rescue on Notch1 levels. (f) Quantification of western blots for Notch1 and ATG16L1/ATG16L1 mStr, relative to actin, normalized for relevant control. *P<0.05 or **P<0.01 by paired t-test. n=3. Error bars=s.e.m. (g,h) HEK cells were transfected with control (scrambled) siRNA/ATG16L1 siRNA +Dll1 ligands (ATG16L1KD), or pcDNA/Beclin-Flag. Notch pathway activity was measured by firefly luciferase reporter assay with RBP-Jκ coupled reporter using Renilla luciferase with constitutive promoter as control. Ratio of sample to control is shown. *P<0.05 and **P<0.01. NS denotes not significant by unpaired t-test. n=3. Error bars=s.e.m. (i) Effect of autophagy on the nuclear translocation of NICD. Scale bar, 20 μm for images of the upper panel. Scale bar, 5 μm for all lower panel images. (j) HEK cells were treated with rapamycin/starved with HBSS, or transfected with pcDNA/Beclin-1. Magnifications of areas in white boxes are shown in lower images and with DAPI in lower panels. Scale bar, 10 μm for upper panels. Scale bar, 5 μm for bottom two rows.

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