

### Anti-ATG7 antibody [EP1759Y] ab52472

KO 評価済 リコンビナント RabMAb

★★★★★ 3 Abreviews 57 References 画像数 10

#### 製品の概要

製品名	Anti-ATG7 antibody [EP1759Y]
製品の詳細	Rabbit monoclonal [EP1759Y] to ATG7
由来種	Rabbit
アプリケーション	適用あり: Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
種交差性	交差種: Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	IHC-P: Human cervical carcinomaWB: Jurkat, HepG2, HEK293 and HAP1 cell lysate ICC/IF: HT-29 and HeLa whole cell lysate ( <b>ab150035</b> )Flow Cyt (intra): HEK293 and HeLa cells
特記事項	<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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb® patents</a>.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

#### 製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	EP1759Y
アイソタイプ	IgG

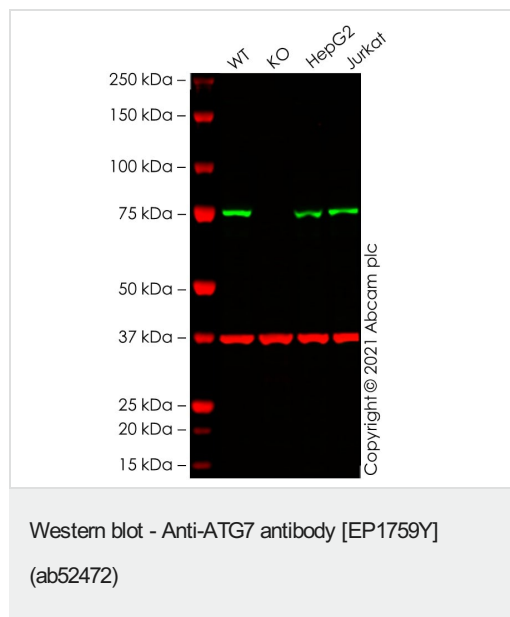
## アプリケーション

**The Abpromise guarantee** Abpromise保証は、次のテスト済みアプリケーションにおけるab52472の使用に適用されます  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/50 - 1/100. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/500. <b>For unpurified use at 10 µg/mL.</b>
WB	★★★★★ (2)	1/100000 - 1/200000. Detects a band of approximately 70 kDa (predicted molecular weight: 78 kDa).
IP		1/30 - 1/50.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

## ターゲット情報

機能	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. 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. Plays also 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.
組織特異性	Widely expressed, especially in kidney, liver, lymph nodes and bone marrow.
配列類似性	Belongs to the ATG7 family.
ドメイン	The C-terminal part of the protein is essential for the dimerization and interaction with ATG3 and ATG12. The N-terminal FAP motif (residues 15 to 17) is essential for the formation of the ATG89-PE and ATG5-ATG12 conjugates.
翻訳後修飾	Acetylated by EP300.
細胞内局在	Cytoplasm. Preautophagosomal structure. Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme.



**All lanes** : Anti-ATG7 antibody [EP1759Y] (ab52472) at 1/100000 dilution

**Lane 1** : Wild-type HeLa cell lysate

**Lane 2** : ATG7 knockout HeLa cell lysate

**Lane 3** : HepG2 cell lysate

**Lane 4** : Jurkat cell lysate

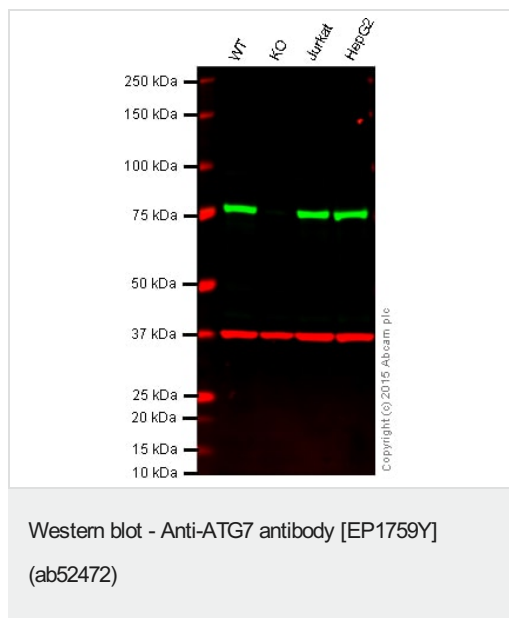
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 78 kDa

**Observed band size:** 75 kDa

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.



**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

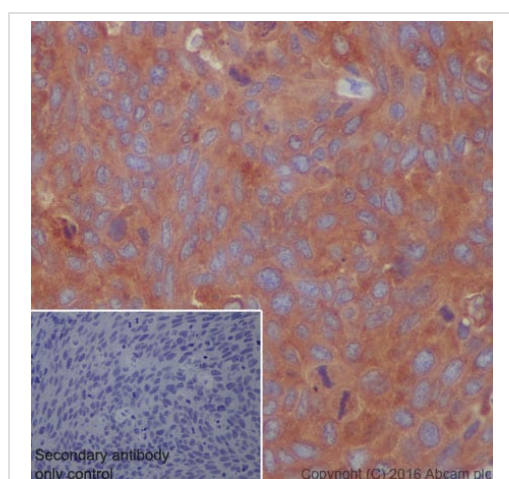
**Lane 2:** ATG7 knockout HAP1 cell lysate (20 µg)

**Lane 3:** Jurkat cell lysate (20 µg)

**Lane 4:** HepG2 cell lysate (20 µg)

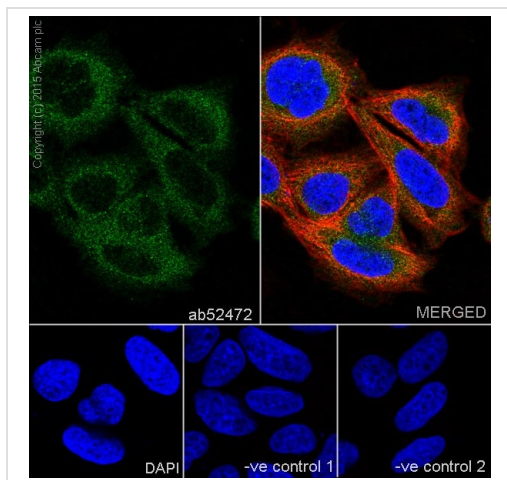
**Lanes 1 - 4:** Merged signal (red and green). Green - ab52472 observed at 77 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

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.



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.

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

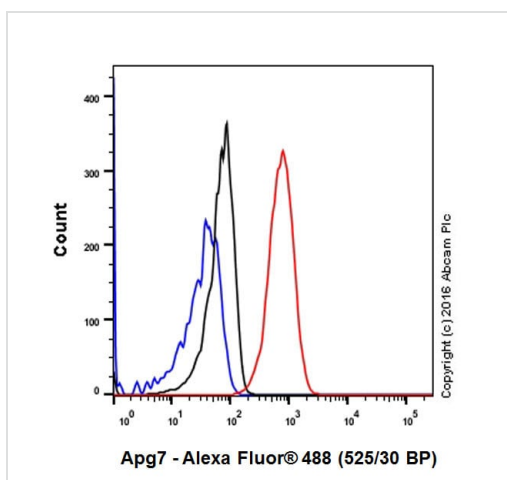


Immunocytochemistry/ Immunofluorescence - Anti-ATG7 antibody [EP1759Y] (ab52472)

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.

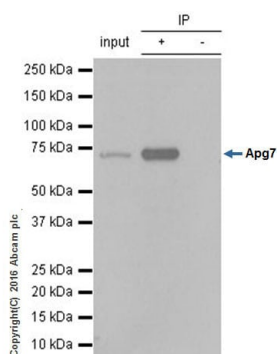
**ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with **ab7291** anti-Tubulin (mouse mAb) followed by **ab150120**, AlexaFluor®594 goat anti-mouse secondary both at 1/1000. Nuclei were counterstained with DAPI (blue).

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**).



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

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.



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).

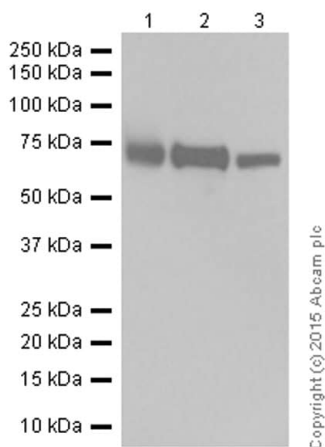
Lane 1 (input): HEK293 whole cell lysate 10ug

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.



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

**All lanes :** Anti-ATG7 antibody [EP1759Y] (ab52472) at 1/100000 dilution

**Lane 1 :** HEK293 whole cell lysate

**Lane 2 :** HepG2 whole cell lysate

**Lane 3 :** Jurkat whole cell lysate

Lysates/proteins at 20 µg per lane.

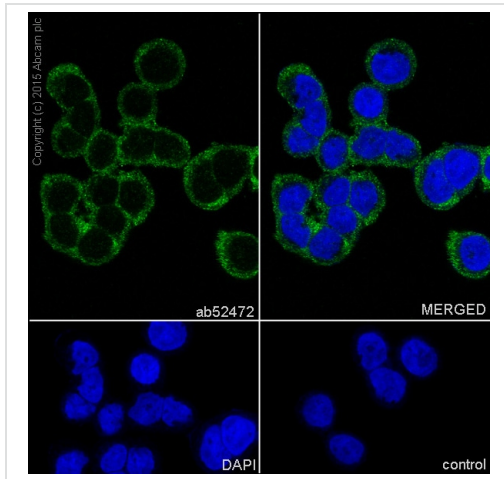
### Secondary

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

**Predicted band size:** 78 kDa

**Observed band size:** 70 kDa

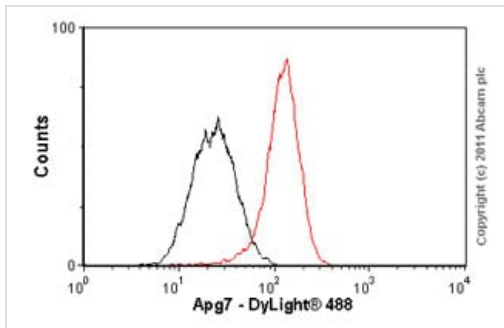
Blocking and Diluting buffer 5% NFDM/TBST



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

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 counterstained 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)

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<sup>6</sup> 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.

### 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-ATG7 antibody [EP1759Y] (ab52472)

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