

Anti-ATG7 antibody [EP1759Y] - BSA and Azide free ab227564

KO 評価済 リコンビナント RabMAb

9 References 画像数 9

製品の概要

製品名	Anti-ATG7 antibody [EP1759Y] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EP1759Y] to ATG7 - BSA and Azide free
由来種	Rabbit
アプリケーション	適用あり: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
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 cell lysate Flow Cyt (intra): HEK293 and HeLa cells
特記事項	<p>ab227564 is the carrier-free version of ab52472.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p>

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.20 Constituent: PBS
キャリア・フリー	はい

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

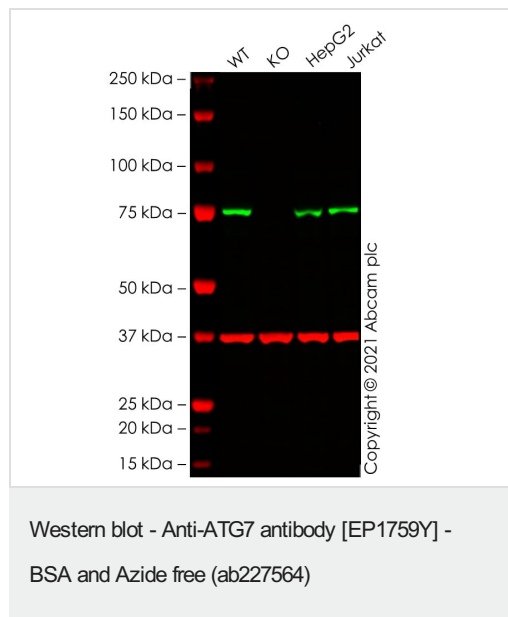
The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab227564の使用に適用されます
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご確認ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
WB		1/100000 - 1/200000. Detects a band of approximately 70 kDa (predicted molecular weight: 78 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

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.

画像



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

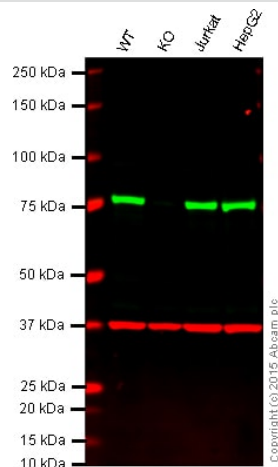
Lysates/proteins at 20 µg per lane.

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.



Western blot - Anti-ATG7 antibody [EP1759Y] - BSA and Azide free (ab227564)

This WB data was generated using the same anti-ATG7 antibody clone, EP1759Y, in a different buffer formulation (cat# [ab52472](#)).

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

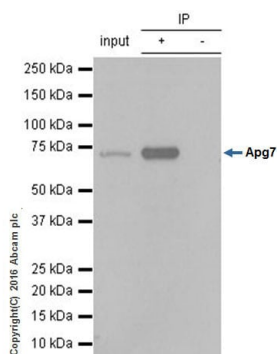
Lane 2: Apg7 knockout HAP1 cell lysate (20 µg)

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 Apg7 when Apg7 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.



Immunoprecipitation - Anti-ATG7 antibody [EP1759Y] - BSA and Azide free (ab227564)

[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

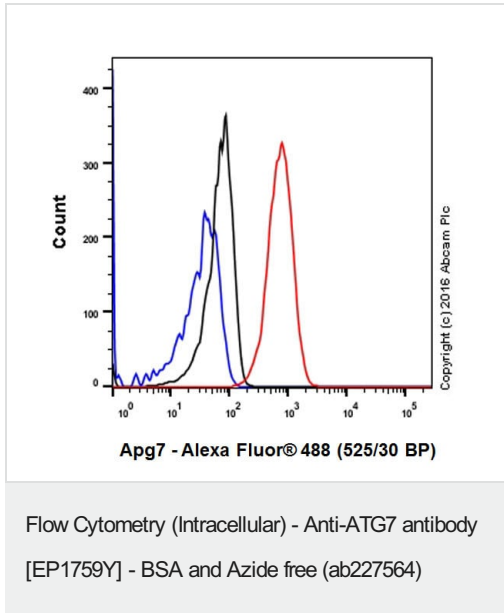
Lane 2 (+): [ab52472](#) + HEK293 whole cell lysate

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 1/10,000 dilution.

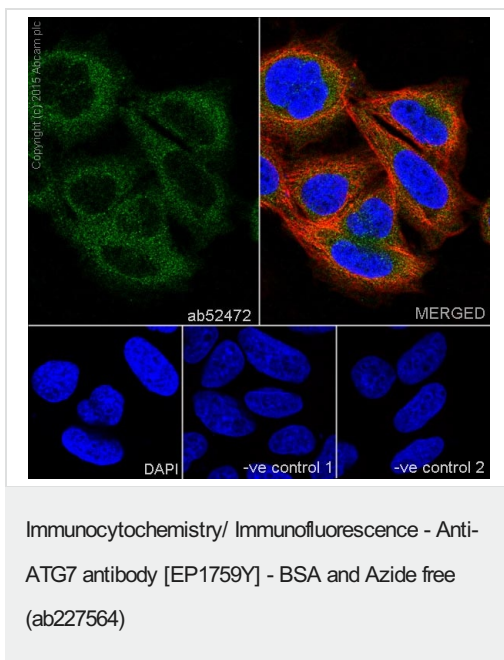
Blocking and Diluting buffer and concentration: 5% NFDm/TBST.

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

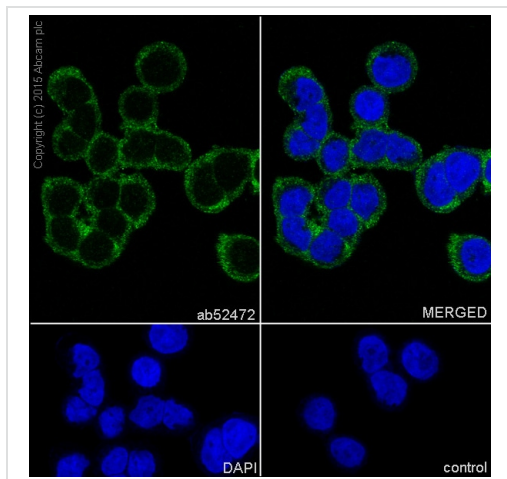
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab52472](#)).



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](#)).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab52472](#)).

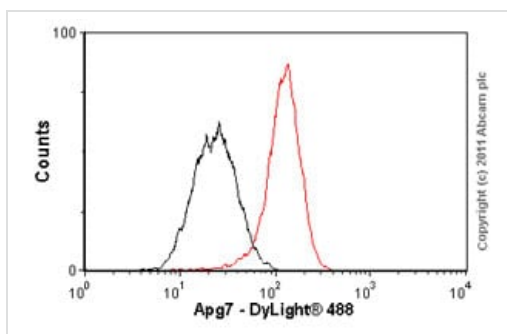


Immunocytochemistry/ Immunofluorescence - Anti-ATG7 antibody [EP1759Y] - BSA and Azide free (ab227564)

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.

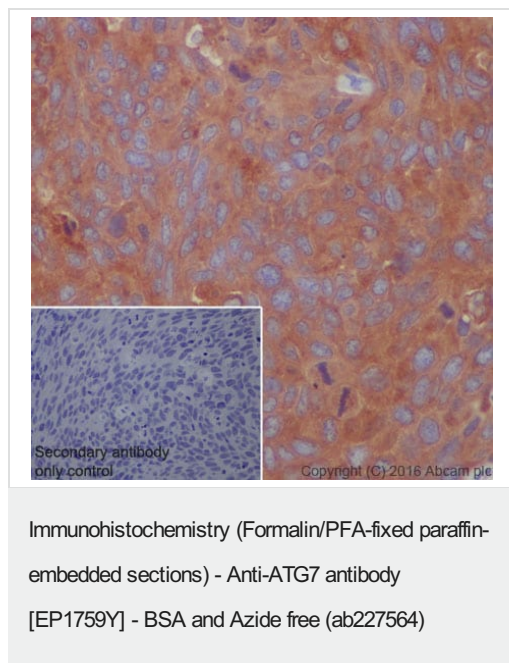
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52472**).



Flow Cytometry (Intracellular) - Anti-ATG7 antibody [EP1759Y] - BSA and Azide free (ab227564)

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/1x10⁶ 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52472**).



This IHC data was generated using the same anti-ATG7 antibody clone, EP1759Y, in a different buffer formulation (cat# **ab52472**.

Immunohistochemical analysis of paraffin-embedded human cervical carcinoma sections labelling Apg7 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.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-ATG7 antibody [EP1759Y] - BSA and Azide free (ab227564)

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