abcam

Product datasheet

Anti-ATG7 antibody [EPR6251] ab133528

敲除验证 重组 RabMAb

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概述

产品名称	Anti-ATG7抗体[EPR6251]
描述	兔单克隆抗体[EPR6251] to ATG7
宿主	Rabbit
经测试应用	**适用于:** WB, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human ATG7 aa 1-100. The exact sequence is proprietary.
阳性对照	293T, HepG2 and Jurkat whole cell lysate (**ab7899**), Rat spleen and kidney tissue lysates, mouse spleen and kidney tissue lysates; HT-29 and HeLa cells.
常规说明	We have had 1 attempt at IHC-P with ab133528 in our own lab. We observed both cytoplasmic and nuclear staining on several tissues (including human stomach, kidney and pancreatic cancer), under our experimental conditions. For IHC-P on human tissues, we would recommend using **ab52472**. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR6251
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab133528于以下的经测试应用**

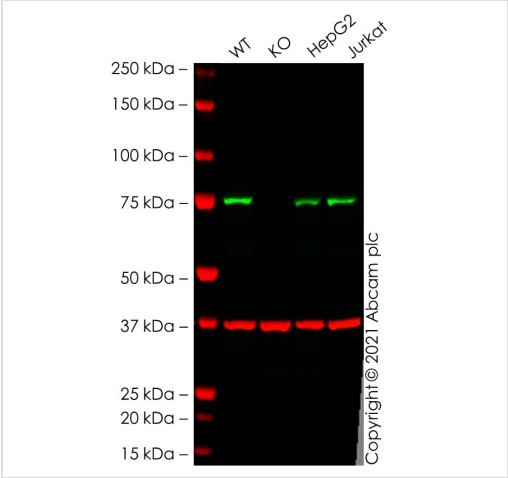
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★☆ (9)	1/10000 - 1/50000. Predicted molecular weight: 77 kDa. Use 5% non-fat dry milk + TBST for blocking.
ICC/IF	★★★★☆ (1)	1/100 - 1/500.

靶标

功能	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.
组织特异性	Widely expressed, especially in kidney, liver, lymph nodes and bone marrow.
序列相似性	Belongs to the ATG7 family.
结构域	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.
翻译后修饰	Acetylated by EP300.
细胞定位	Cytoplasm. Preautophagosomal structure. Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme.

图片



Western blot - Anti-ATG7 antibody [EPR6251] (ab133528)

All lanes : Anti-ATG7 antibody [EPR6251] (ab133528) at 1/10000 dilution

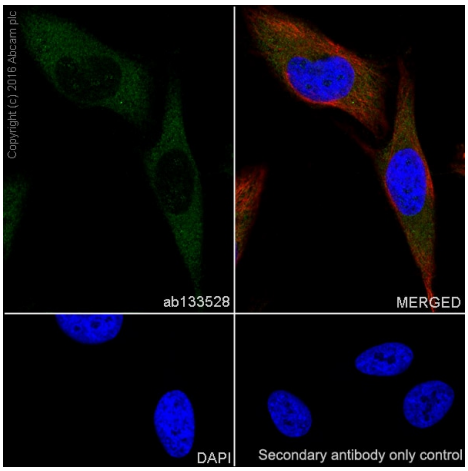
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG7 knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

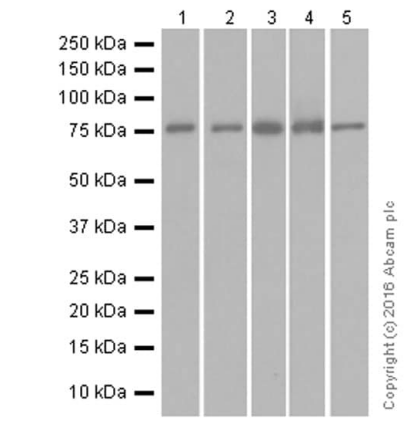
Predicted band size: 77 kDa
Observed band size: 75 kDa

False colour image of Western blot: Anti-ATG7 antibody [EPR6251] staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133528 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.



Immunocytochemistry/ Immunofluorescence - Anti-ATG7 antibody [EPR6251] (ab133528)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling ATG7 with purified ab133528 at 1/150 dilution (8.5µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1/1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-ATG7 antibody [EPR6251] (ab133528)

All lanes : Anti-ATG7 antibody [EPR6251] (ab133528) at 1/50000 dilution (purified)

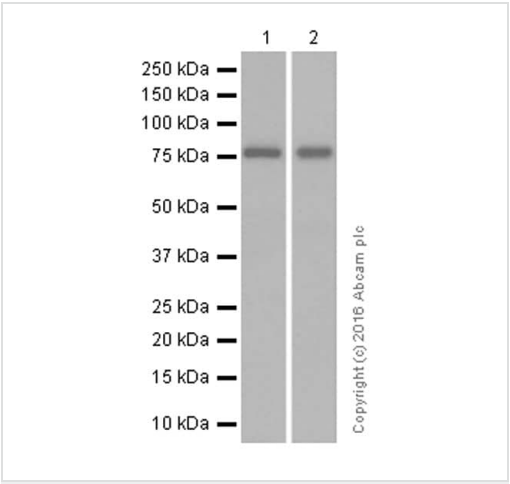
Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 2 : Mouse spleen lysate
Lane 3 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 4 : Mouse kidney lysate
Lane 5 : Rat kidney lysate

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: 77 kDa
Observed band size: 77 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-ATG7 antibody [EPR6251] (ab133528)

All lanes : Anti-ATG7 antibody [EPR6251] (ab133528) at 1/10000 dilution (purified)

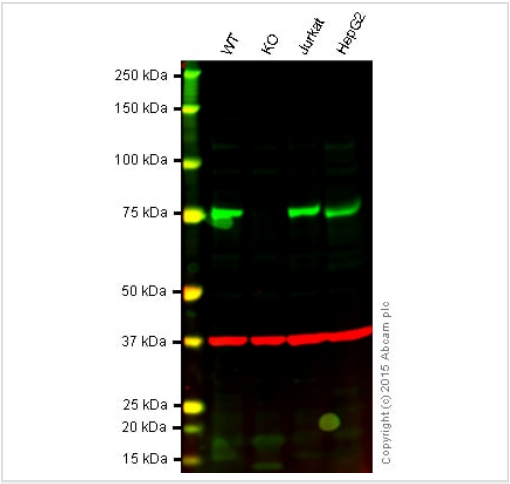
Lane 1 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 2 : Rat spleen lysate

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: 77 kDa
Observed band size: 77 kDa

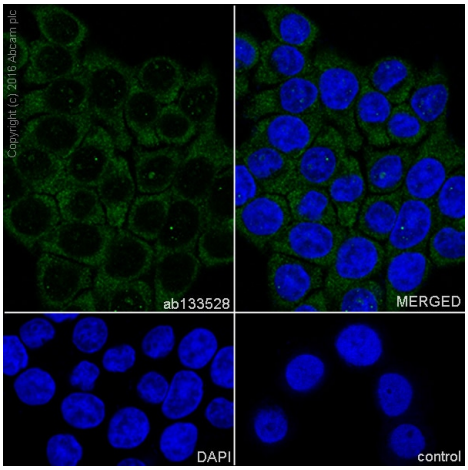
Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-ATG7 antibody [EPR6251] (ab133528)

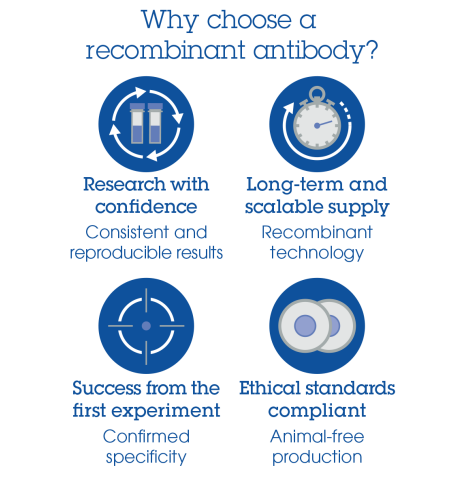
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: ATG7 knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab133528 observed at 77 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab133528 was shown to specifically react with ATG7 when ATG7 knockout samples were used. Wild-type and Apg7 knockout samples were subjected to SDS-PAGE. ab133528 and **ab8245** (loading control to GAPDH) were diluted 1/10,000 and 1/2000 respectively 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 1/10 000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ATG7 antibody [EPR6251] (ab133528)

Immunocytochemistry/Immunofluorescence analysis of HT-29 (Human colorectal adenocarcinoma cell line) labeling ATG7 with purified ab133528 at 1/500 dilution. Cells were fixed with 100% methanol. **ab150077** Goat anti rabbit IgG (Alexa Fluor® 488) at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.



Anti-ATG7 antibody [EPR6251] (ab133528)

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