Product datasheet

Anti-ATG7 antibody [EPR6251] ab133528

**Overview**

**Product name**  Anti-ATG7 antibody [EPR6251]

**Description**  Rabbit monoclonal [EPR6251] to ATG7

**Host species**  Rabbit

**Tested applications**  Suitable for: WB, ICC/IF

**Species reactivity**  Reacts with: Mouse, Rat, Human

**Immunogen**  Synthetic peptide within Human ATG7 aa 1-100. The exact sequence is proprietary.

**Positive control**  293T, HepG2 and Jurkat whole cell lysate (ab7899), Rat spleen and kidney tissue lysates; HT-29 and HeLa cells.

**General notes**  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.

**Properties**

**Form**  Liquid

**Storage instructions**  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.

**Storage buffer**  pH: 7.2
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

**Purity**  Protein A purified
Clonality: Monoclonal
Clone number: EPR6251
Isotype: IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab133528 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★☆☆ (9)</td>
<td>1/10000 - 1/50000. Predicted molecular weight: 77 kDa. Use 5% non-fat dry milk + TBST for blocking.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★☆☆ (1)</td>
<td>1/100 - 1/500.</td>
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Target

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. 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.

Tissue specificity
Widely expressed, especially in kidney, liver, lymph nodes and bone marrow.

Sequence similarities
Belongs to the ATG7 family.

Domain
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.

Post-translational modifications
Acetylated by EP300.

Cellular localization
Cytoplasm. Preautophagosomal structure. Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme.

Images
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 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.

**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.
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.

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 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.

**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 [EPR6251] (ab133528)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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