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 cell lysates, Rat spleen and kidney tissue lysates, mouse 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.
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

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: 7.40
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

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>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/10000 - 1/50000. Predicted molecular weight: 77 kDa. Use 5% non-fat dry milk + TBST for blocking.</td>
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<tr>
<td>ICC/IF</td>
<td>🌟🌟🌟🌟🌟</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.
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.
**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.

**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.

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