abcam

Product datasheet

Autophagy Marker (APG5L/ATG5, ATG16L1, ATG4B, ATG9A, Beclin 1, LC3B) Antibody Sampler Panel ab228525

Recombinant

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Overview

Product name

Autophagy Marker (APG5L/ATG5, ATG16L1, ATG4B, ATG9A, Beclin 1, LC3B) Antibody

Sampler Panel

Species reactivity

Product overview

Reacts with: Mouse, Rat, Human

Autophagy Antibody Panel Sampler Panel (ab228525) contains multiple trial-sized versions of clones against APG5L/ATG5, ATG16L1, ATG4B, ATG9A, Beclin 1, and LC3B specifically selected for high performance in various applications. This panel contains 6 recombinant rabbit monoclonal antibodies, 5 of which are knock-out validated. They are provided as a sampler panel to allow you to easily evaluate each in your required applications.

For guidelines on how to use each antibody within the panel, please consult the individual datasheet for each antibody.

Panel contains:

- Rabbit monoclonal [EPR1755(2)] to APG5L/ATG5 (20 μL) ab108327
- Rabbit monoclonal [EPR15638] to ATG16L1 N-terminal (20 µL) ab187671
- Rabbit monoclonal [EPR6436(2)] to ATG4B (20 µL) ab154843
- Rabbit monoclonal [EPR2450(2)] to ATG9A (20 $\mu L)$ $\underline{ab108338}$
- Rabbit monoclonal [EPR19662] to Beclin 1 (20 μL) **ab207612**
- Rabbit monoclonal [EPR18709] to LC3B (20 μL) ab192890

Explore our range of antibody sample panels designed to provide you with a variety of trial-size antibodies in a convenient and cost-effective format.

Notes

Directly conjugated versions of our antibodies are available and ready to use for multicolor flow cytometry or immunocytochemistry analysis. **Carrier-free formulations** are also available for easy conjugation to labels of your choice. Please refer to the 'Associated products' section below.

Tested applications

Suitable for: WB

Properties

Storage instructions

Store at -20°C. Please refer to protocols.

| Components | 1 kit |
|--|----------|
| ab108327 - Anti-APG5L/ATG5 antibody [EPR1755(2)] | 2 x 10µl |
| ab187671 - Anti-ATG16L1 antibody [EPR15638] | 2 x 10µl |
| ab154843 - Anti-ATG4B antibody [EPR6436(2)] | 2 x 10µl |
| ab108338 - Anti-ATG9A antibody [EPR2450(2)] | 2 x 10µl |
| ab207612 - Anti-Beclin 1 antibody [EPR19662] | 2 x 10µl |
| ab192890 - Anti-LC3B antibody [EPR18709] | 2 x 10µl |

Cellular localization

Beclin 1: Golgi apparatus > trans-Golgi network membrane. Interaction with ATG14 promotes translocation to autophagosomes. Expressed in dendrites and cell bodies of cerebellar Purkinje cells. APG5L/ATG5: Cytoplasm. Preautophagosomal structure membrane. Colocalizes with nonmuscle actin. The conjugate detaches from the membrane immediately before or after autophagosome formation is completed (By similarity). Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme. ATG16L1: Cytoplasm. Preautophagosomal structure membrane. Recruited to omegasomes membranes by WIPI2. Omegasomes are endoplasmic reticulum connected strutures at the origin of preautophagosomal structures. Localized to preautophagosomal structure (PAS) where it is involved in the membrane targeting of ATG5. Localizes also to discrete punctae along the ciliary axoneme. ATG9A: Cytoplasmic vesicle, autophagosome membrane. Golgi apparatus, trans-Golgi network membrane. Late endosome membrane. Endoplasmic reticulum membrane. Under amino acid starvation or rapamycin treatment, redistributes from a juxtanuclear clustered pool to a dispersed peripheral cytosolic pool. The starvation-induced redistribution depends on ULK1, ATG13, as well as SH3GLB1. LC3B: Cytoplasm > cytoskeleton. Endomembrane system. Cytoplasmic vesicle > autophagosome membrane. LC3-II binds to the autophagic membranes. ATG4B: Cytoplasm.

Applications

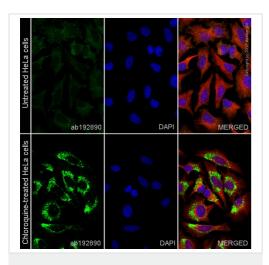
The Abpromise guarantee

Our Abpromise guarantee covers the use of ab228525 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|-------------|-----------|--|
| WB | | Use at an assay dependent concentration. |

Images



Anti-LC3B antibody [EPR18709]

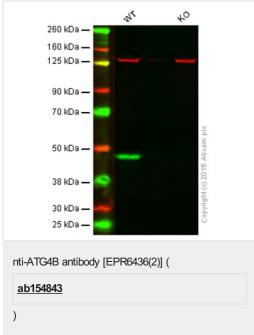


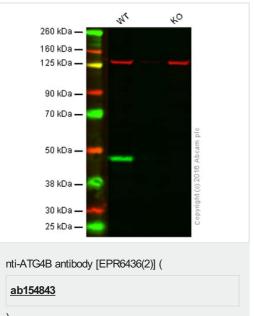
ab192890 staining LC3B in HeLa cells +/- Chloroquine (50μM, 24 hours). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab192890 at 1μg/ml and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red) followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit lgG (Alexa Fluor® 488) (ab150081) at 2 μg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

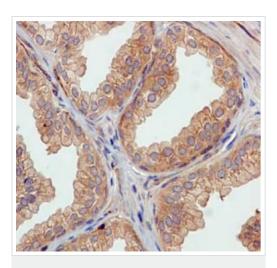
<u>ab108338</u>, at 1/100, staining ATG9A in paraffin-embedded Human colon tissue by Immunohistochemistry.



Immunocytochemistry/ Immunofluorescence analysis of HeLa cells labeling Beclin 1 with <u>ab207612</u> at 1/200 dilution. Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton-X100 in PBS. Samples were incubated with primary antibody (1/200 in PBS) for 16 hours at 22°C. <u>ab150081</u>, a goat anti-rabbit lgG H + L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution.







Anti-ATG16L1 [EPR15638] antibody

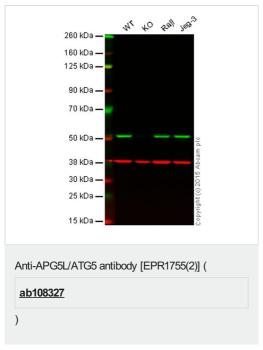
Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: ATG4B knockout HAP1 cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - ab154843 observed at 47 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab154843 was shown to specifically react with ATG4B when ATG4B knockout samples were used. Wild-type and ATG4B knockout samples were subjected to SDS-PAGE. ab154843 and ab18058 (loading control to Vinculin) were diluted at 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit lgG 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 hour at room temperature before imaging.

Immunohistochemical analysis of paraffin-embedded Human prostatic hyperplasia tissue labeling ATG16L1 with ab187671 at 1/100 dilution followed by pre-diluted HRP Polymer for Rabbit IgG secondary antibody and counter-stained with Hematoxylin.



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

Lane 2: APG5L/ATG5 knockout HAP1 cell lysate (20 μg)

Lane 3: Raji cell lysate (20 μg)

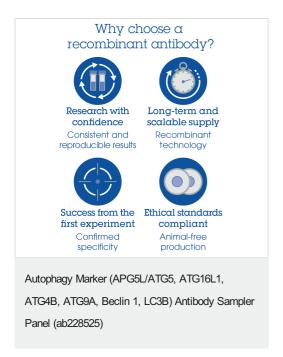
Lane 4: Jeg-3 cell lysate (20 μg)

Lanes 1 - 4: Merged signal (red and green). Green - ab108327

observed at 52 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab108327 was shown to specifically react with APG5L/ATG5 when APG5L/ATG5 knockout samples were used. Wild-type

when APG5L/ATG5 knockout samples were used. Wild-type and APG5L/ATG5 knockout samples were subjected to SDS-PAGE. ab108327 and ab245 (loading control to GAPDH) were both diluted 1/1000 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.



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

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