abcam

Product datasheet

Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free ab223528

KO VALIDATED Recombinant RabMAb

7 References 9 Images

Overview

Product name	Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR2450(2)] to ATG9A - BSA and Azide free
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, IP, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HepG2, 293T, A375, cell line lysates Mouse brain and rat brain cell lysates Paraffin-embedded human colon tissue
General notes	ab223528 is the carrier-free version of **ab108338**. 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. 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. 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. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2450(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab223528 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 94 kDa.
ICC/IF		Use at an assay dependent concentration.

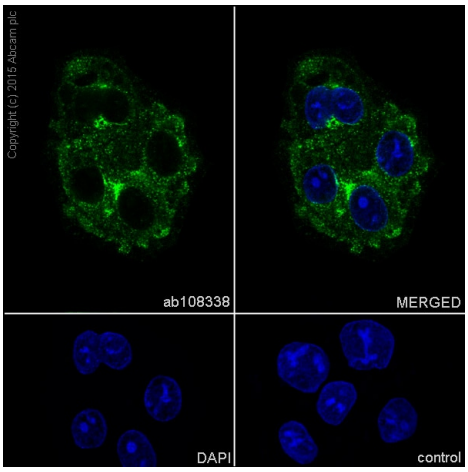
Target

Function	Involved in autophagy and cytoplasm to vacuole transport (Cvt) vesicle formation. Plays a key role in the organization of the preautophagosomal structure/phagophore assembly site (PAS), the nucleating site for formation of the sequestering vesicle. Cycles between a juxta-nuclear trans-Golgi network compartment and late endosomes. Nutrient starvation induces accumulation on autophagosomes. Starvation-dependent trafficking requires ULK1, ATG13 and SUPT20H.
Sequence similarities	Belongs to the ATG9 family.

Cellular localization

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.

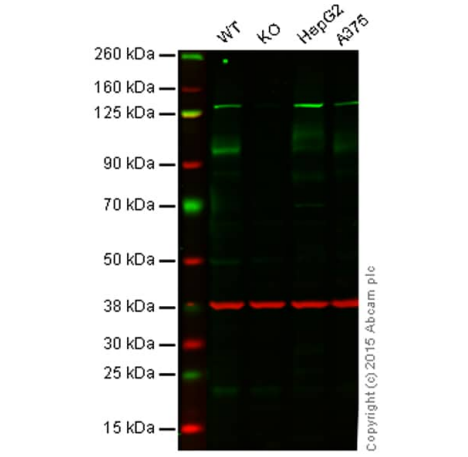
Images



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

Immunocytochemistry/ Immunofluorescence analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with Purified **ab108338** at 1:100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. **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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108338**).

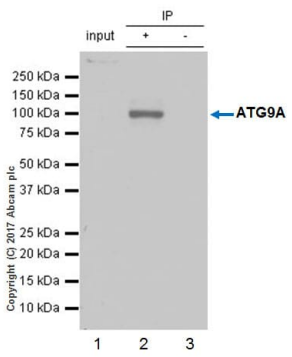


Western blot - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

This western blot data was generated using the same anti-ATG9A clone (EPR2450(2)] in a different buffer formulation (cat# **ab108338**).

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: ATG9A knockout HAP1 cell lysate (20 µg)
Lane 3: HepG2 cell lysate (20 µg)
Lane 4: A375 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - **ab108338** observed at 100 and 130 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab108338 was shown to specifically react with ATG9A when ATG9A knockout samples were used. Wild-type and ATG9A knockout samples were subjected to SDS-PAGE. **ab108338** and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 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 1/10,000 dilution for 1 h at room temperature before imaging.



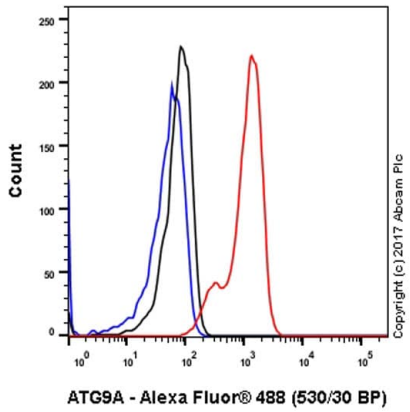
Immunoprecipitation - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

ab108338 (purified) at 1:20 dilution (2µg) immunoprecipitating ATG9A in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate.

Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab108338 & HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab108338** in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution. No band in input lane is due to the boiled lysates
Blocking and diluting buffer: 5% NFDM/TBST.

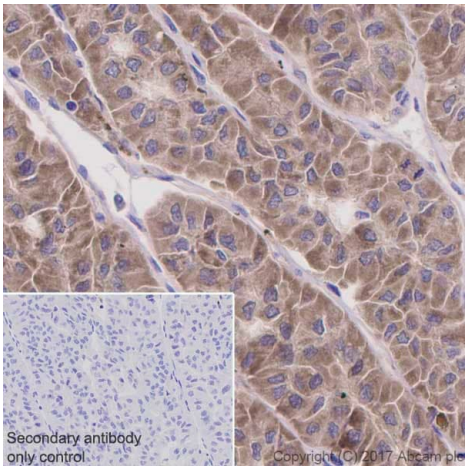
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108338**).



Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with purified **ab108338** at 1/20 dilution (10µg/ml) (red). Cells were fixed with 100% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

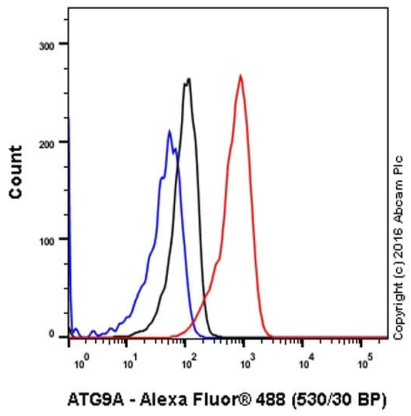
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108338**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human thyroid carcinoma tissue sections labeling ATG9A with Purified **ab108338** at 1:50 dilution (4.12 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used 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 (**ab108338**).



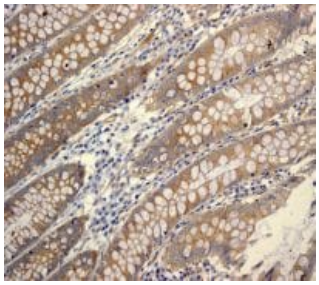
Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

Unpurified **ab108338** staining ATG9A in the human cell line HepG2 (human hepatocellular carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/40. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108338**).

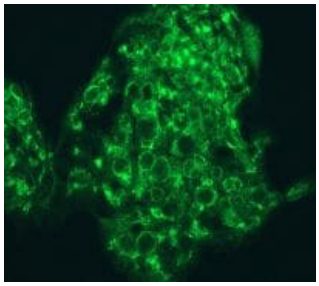


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

Unpurified **ab108338**, at 1/100, staining ATG9A in paraffin-embedded Human colon tissue by Immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108338**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

Unpurified **ab108338** at 1/50 dilution, staining ATG9A in HepG2 cells by Immunofluorescence.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108338**).

Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

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