abcam

Product datasheet

Anti-ATG9A antibody [EPR2450(2)] ab108338

KO VALIDATED Recombinant RabMAb

14 Abreviews 71 References 14 Images

Overview

Product name	Anti-ATG9A antibody [EPR2450(2)]
Description	Rabbit monoclonal [EPR2450(2)] to ATG9A
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), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HepG2, 293T, A375, cell line lysates; Mouse brain and rat brain cell lysates IHC-P: Paraffin-embedded human colon tissue; Human thyroid carcinoma tissue. ICC/IF: HepG2 cells.
General notes	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**. **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.**

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2450(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108338 in the following tested applications.

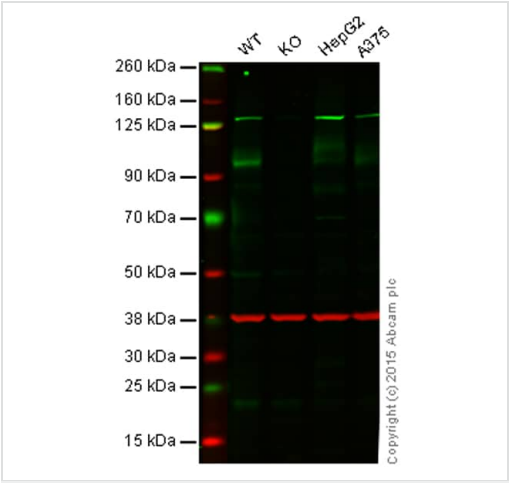
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
WB	★★★★★ **(8)**	1/1000. Predicted molecular weight: 94 kDa.
IP		1/10 - 1/100.
IHC-P		1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat. **For unpurified use 1/100 - 1/250.**
ICC/IF	★★★★★ **(5)**	1/50 - 1/100.

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.

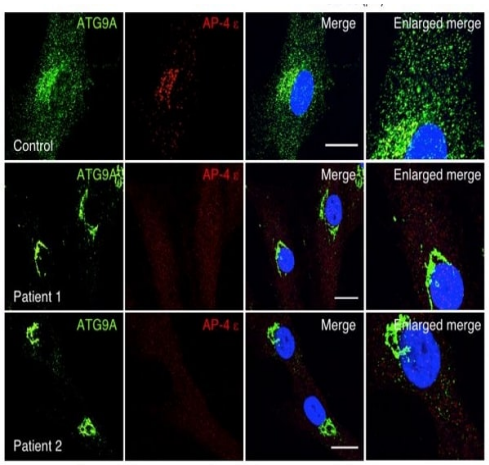
Images



Western blot - Anti-ATG9A antibody [EPR2450(2)] (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.

Unpurified 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.



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

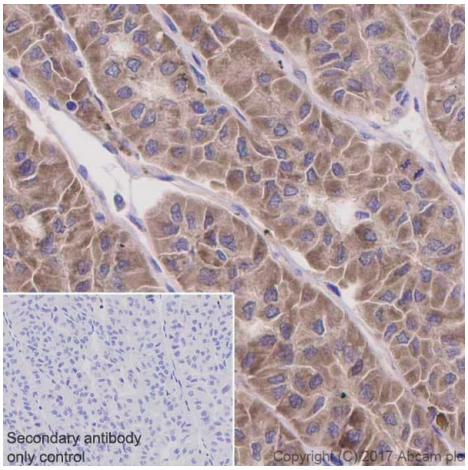
Accumulation of ATG9A at the TGN of AP-4 µ4 mutant patient fibroblasts.

Skin fibroblasts were from one control individual and two patients homozygous for mutations in the AP4M1 gene encoding AP-4 µ4.

Co-immunostaining for endogenous ATG9A (green) and AP-4 ε (red) (B) or GM130 of the fibroblasts.

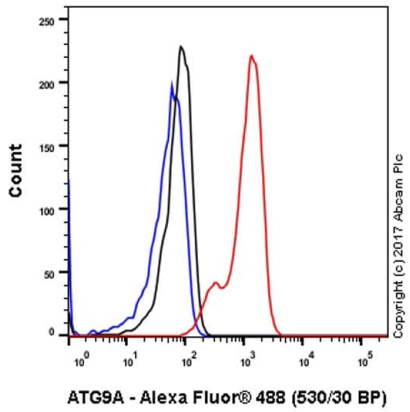
ATG9A is detected using ab108338.

(From Figure 4B of De Pace et al)



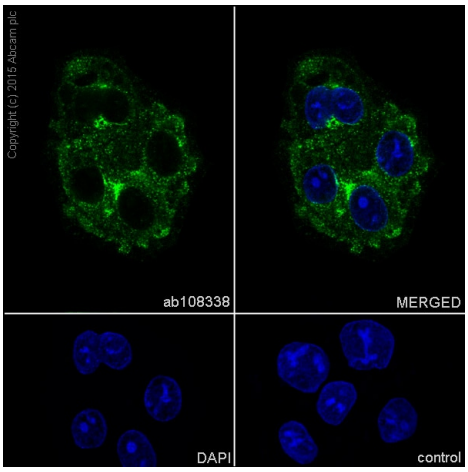
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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.



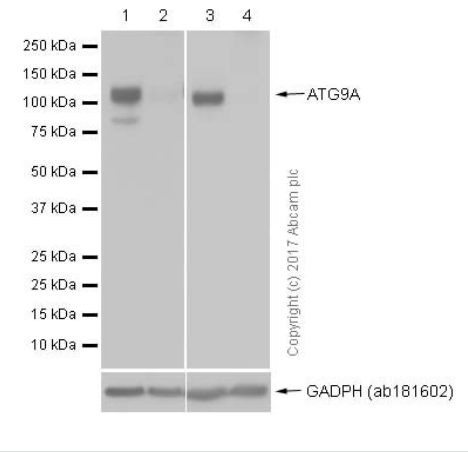
Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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).



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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.



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

All lanes : Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/10000 dilution (purified)

Lane 1 : 293 (Human embryonic kidney epithelial cell) whole cell lysate prepared in non-boiled method
Lane 2 : 293 (Human embryonic kidney epithelial cell) whole cell lysate prepared in boiled method
Lane 3 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared in non-boiled method
Lane 4 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared in boiled method

Lysates/proteins at 15 µg per lane.

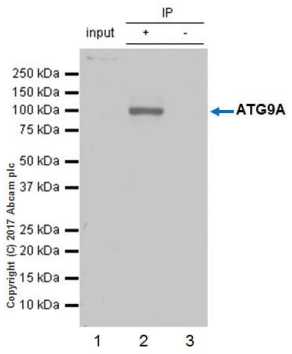
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 94 kDa
Observed band size: 100 kDa

We suggest not to boil the sample after lysis.

Blocking and diluting buffer: 5% NFDM/TBST

Exposure time:
Left image: 5 seconds
Right image: 2 seconds

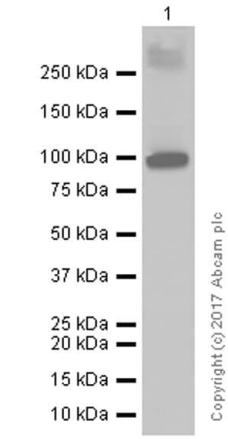


Immunoprecipitation - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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.



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

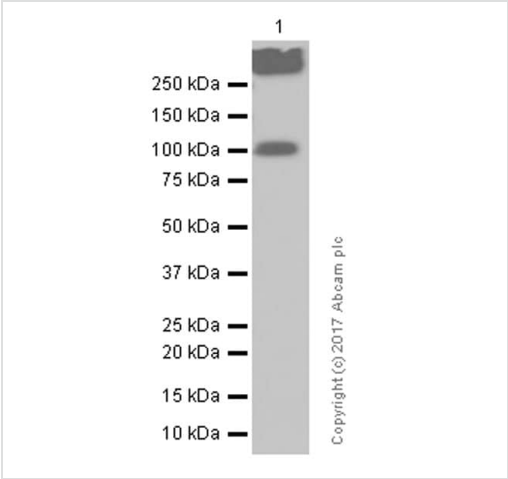
Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/2000 dilution (purified) + Mouse spinal cord lysates at 15 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 94 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

The lysates are boiled.



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/2000 dilution (purified) + Rat brain lysates at 15 µg

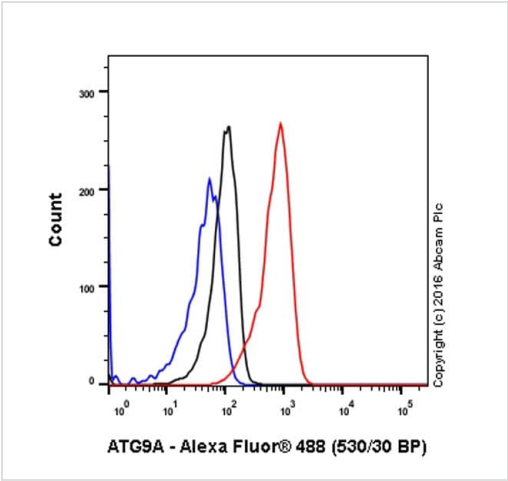
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 94 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

The lysates are boiled.

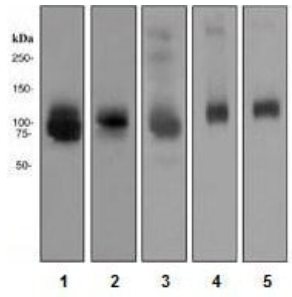


Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

Unpurified ab108338 staining ATG9Ain 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)



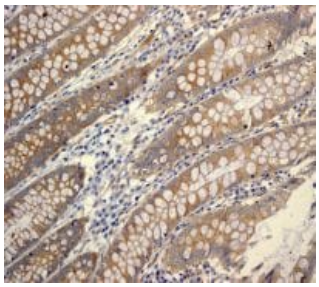
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

All lanes : Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/1000 dilution (unpurified)

Lane 1 : HepG2 cell lysate
Lane 2 : 293T cell lysate
Lane 3 : A375 cell lysate
Lane 4 : Mouse brain cell lysate
Lane 5 : Rat brain cell lysate

Lysates/proteins at 10 µg per lane.

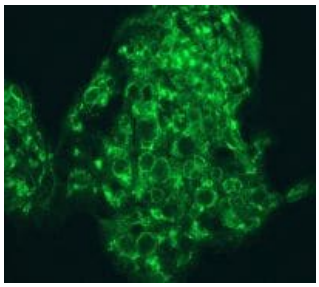
Predicted band size: 94 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

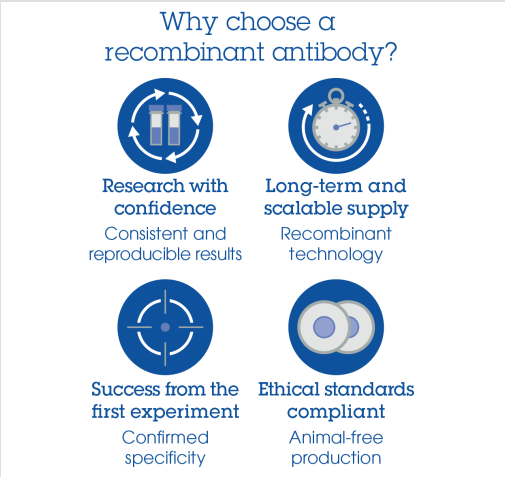
Unpurified ab108338, at 1/100, staining ATG9A in paraffin-embedded Human colon tissue by Immunohistochemistry.

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



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

Unpurified ab108338 at 1/50 dilution, staining ATG9A in HepG2 (Human hepatocellular carcinoma epithelial cell) cells by Immunofluorescence.



Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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