abcam

Product datasheet

Anti-ATG9A antibody [EPR2450(2)] ab108338

敲除验证 重组 RabMAb

14 Abreviews 71 References 14 图像

概述

产品名称	Anti-ATG9A抗体[EPR2450(2)]
描述	兔单克隆抗体[EPR2450(2)] to ATG9A
宿主	Rabbit
特异性	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
经测试应用	**适用于:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR2450(2)
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab108338于以下的经测试应用**

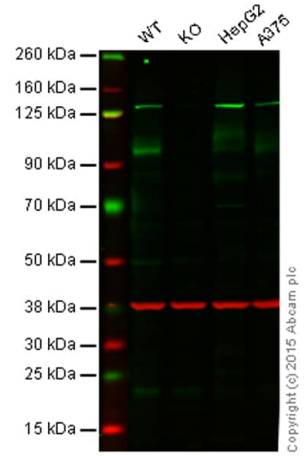
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标

功能	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.
序列相似性	Belongs to the ATG9 family.
细胞定位	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.

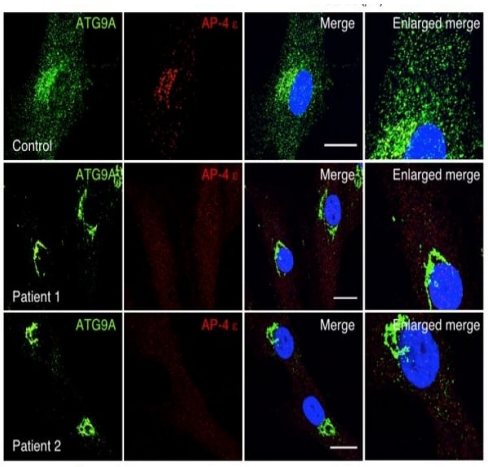
图片



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)

De Pace, R. et al PLoS Genet. 2018 Apr 26;14(4):e1007363. doi: 10.1371/journal.pgen.1007363. eCollection 2018 Apr

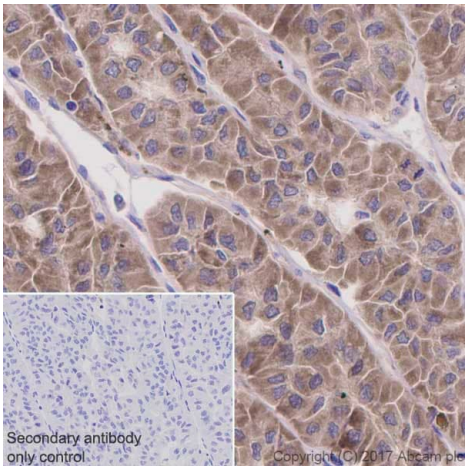
Accumulation of ATG9A at the TGN of AP-4 µ4 mutant patient fibroblasts.

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.

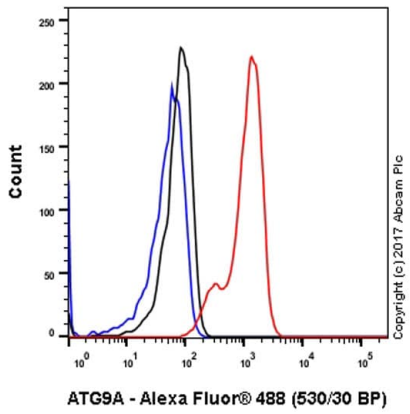
ATG9A is detected using ab108338.

(From Figure 4B of De Pace et al)



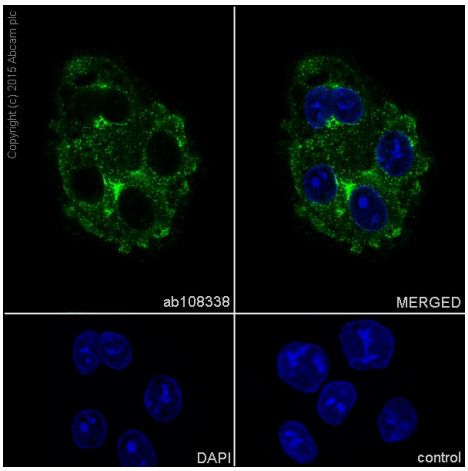
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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.



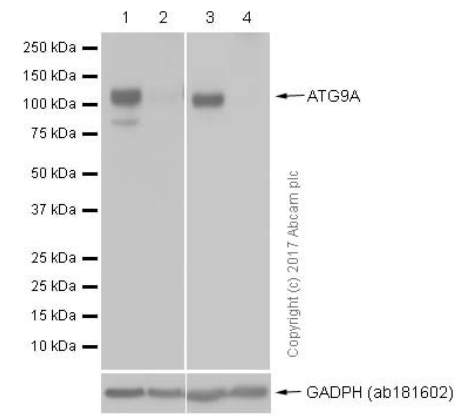
Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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

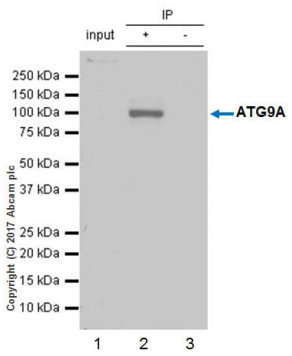
Lysates/proteins at 15 µg per lane.

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

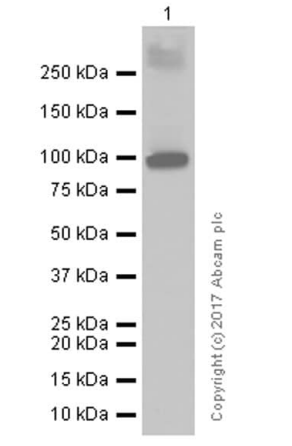


Immunoprecipitation - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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)

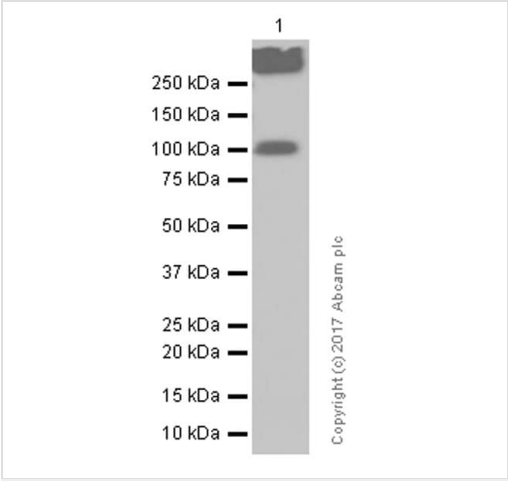
Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/2000 dilution (purified) + Mouse spinal cord lysates at 15 µg

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

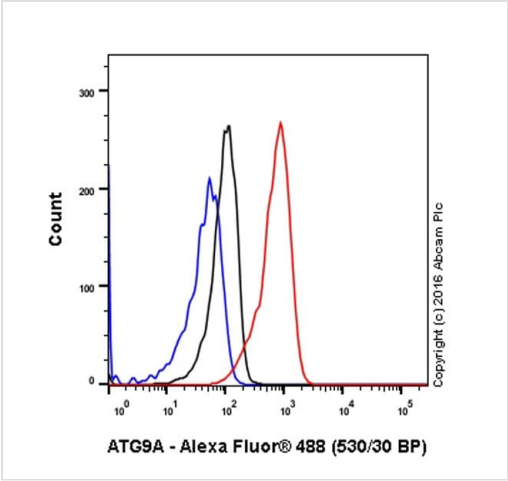
Secondary

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.

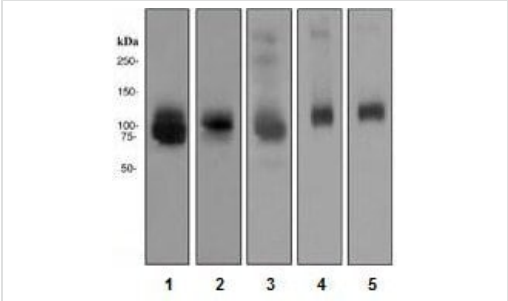


Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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)



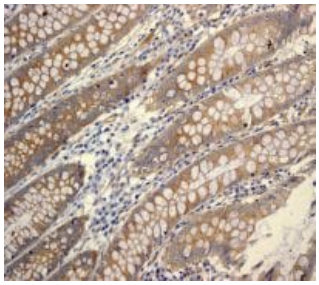
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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.

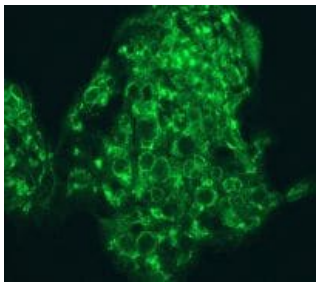
Predicted band size: 94 kDa



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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