abcam

Product datasheet

Anti-ATG9A antibody [EPR2450(2)] ab108338



ילעבע RabMAb

★★★★★ 14 Abreviews 71 References 画像数 14

製品の概要

製品名 Anti-ATG9A antibody [EPR2450(2)]

製品の詳細 Rabbit monoclonal [EPR2450(2)] to ATG9A

由来種 Rabbit

特異性 The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

アプリケーション 適用あり: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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**.

製品の特性

製品の状態

保存方法 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.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 59% PBS, 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル

アイソタイプ

ΙgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab108338の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

機能

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.

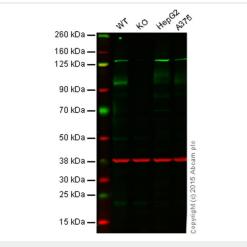
配列類似性

Belongs to the ATG9 family.

細胞内局在

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.

画像



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

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 Reproduced under the Creative Commons license https://creativecommons.org/publicdomain/zero/1.0/

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.

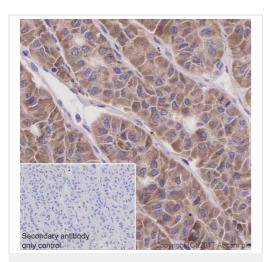
Unpurified ab108338 was shown to specifically react with ATG9A when ATG9A knockout samples were used. Wild-type and ATG9A knockout samples were subjected to SDS-PAGE. ab108338 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 and incubated overnight at 4°C. Blots were developed with 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.

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.

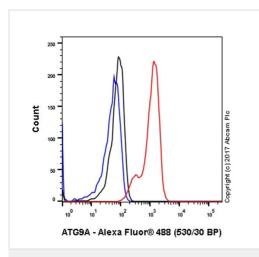
ATG9A is detected using ab108338.

(From Figure 4B of De Pace et al)



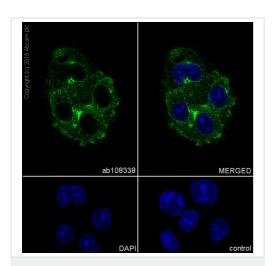
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ATG9A antibody
[EPR2450(2)] (ab108338)

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.



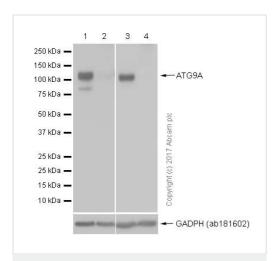
Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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 lgG (Alexa Fluor[®] 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (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) (<u>ab97051</u>) 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:

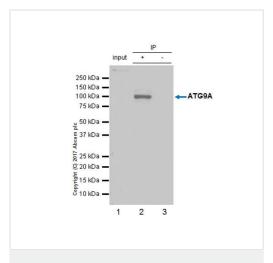
cell lysate.

Left image: 5 seconds Right image: 2 seconds

whole cell lysate 10µg

whole cell lysate

epithelial cell) whole cell lysate



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

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

ab108338 in HEK-293 (Human embryonic kidney epithelial cell)

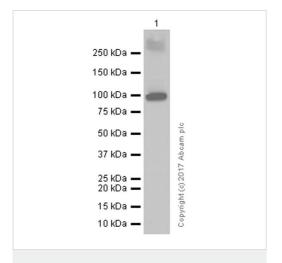
ab108338 (purified) at 1:20 dilution (2µg) immunoprecipitating ATG9A in HEK-293 (Human embryonic kidney epithelial cell) whole

Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell)

Lane 2 (+): ab108338 & HEK-293 (Human embryonic kidney

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of

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

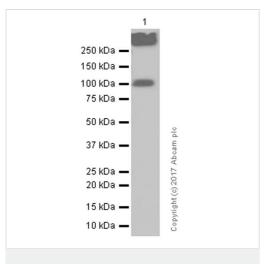
Secondary

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

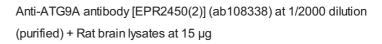
Predicted band size: 94 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

The lysates are boiled.



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



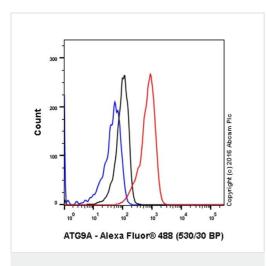
Secondary

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.

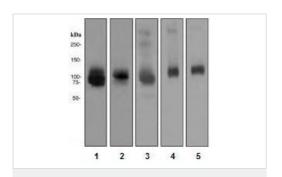


Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

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 lgG (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)

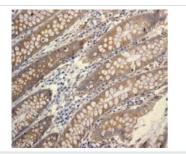
Lane 1 : HepG2 cell lysate
Lane 2 : 293T cell lysate
Lane 3 : A375 cell lysate

Lane 4 : Mouse brain cell lysate

Lane 5 : Rat brain cell lysate

Lysates/proteins at 10 µg per lane.

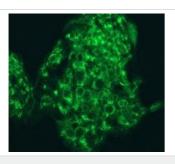
Predicted band size: 94 kDa



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

Unpurified ab108338, at 1/100, staining ATG9A in paraffinembedded 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.



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