abcam

ab223528

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

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR2450(2)
Purification technique	Affinity purification Protein A
Concentration	1.042 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information ATG9A

Function Phospholipid scramblase involved in autophagy by mediating autophagosomal membrane expansion (PubMed:22456507, PubMed:27510922, PubMed:29437695, PubMed:32513819, PubMed:32610138, PubMed:33106659, PubMed:33468622, PubMed:33850023). Cycles between the preautophagosomal structure/phagophore assembly site (PAS) and the cytoplasmic vesicle pool and supplies membrane for the growing autophagosome (PubMed:16940348, PubMed:22456507, PubMed:33106659). Lipid scramblase activity plays a key role in preautophagosomal structure/phagophore assembly by distributing the phospholipids that arrive through ATG2 (ATG2A or ATG2B) from the cytoplasmic to the luminal leaflet of the bilayer, thereby driving autophagosomal membrane expansion (PubMed:33106659). Also required to supply phosphatidylinositol 4-phosphate to the autophagosome initiation site by recruiting the phosphatidylinositol 4-kinase beta (PI4KB) in a process dependent on ARFIP2, but not ARFIP1 (PubMed:30917996). In addition to autophagy, also plays a role in necrotic cell death (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab223528 is the carrier-free version of ab108338.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	ATG9A also known as APG9-like 1 or ATG9 autophagy related 9A is an important component in the autophagy machinery. This protein has a molecular weight of approximately 93 kDa. It is ubiquitously expressed in various tissues with higher

	expression seen in the heart and skeletal muscle. Mechanically ATG9A is essential for the trafficking of membranes necessary for autophagosome formation. It actively participates in membrane lipids' delivery from donor organelles which are critical for constructing autophagosomes.
Biological function summary	ATG9A facilitates the recycling of cellular materials through a process called autophagy. This protein acts with other autophagy-related proteins to form a complex essential for autophagosome elongation and closure. The ATG9A protein's dynamic movements between the Golgi apparatus and endosomes ensure proper membrane supply for autophagy. It coordinates with the ULK1 complex and various phospholipid-modifying enzymes which are necessary to regulate autophagic flux.
Pathways	ATG9A plays an integral role in the mTOR signaling and MAPK pathways. Both pathways are essential for cellular responses to stress and nutrient availability. In these pathways ATG9A works closely with proteins like beclin 1 and ATG5. Inhibiting mTOR actively induces autophagy where ATG9A contributes to membrane recruitment and elongation of the autophagosomes facilitating the clearance of damaged organelles and proteins.
Associated diseases and disorders	ATG9A shows a significant link to neurodegenerative diseases such as Parkinson's and Alzheimer's disease. The dysfunction of autophagic pathways due to altered ATG9A function leads to the accumulation of damaged proteins and organelles. This event contributes to the pathogenesis of such neurodegenerative diseases. Moreover ATG9A closely associates with proteins like LAMP2 whose mutations similarly lead to defective autophagic processes resulting in the accumulation of unprocessed cellular debris in these disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

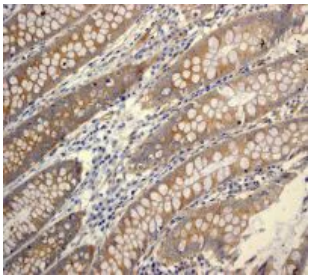
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

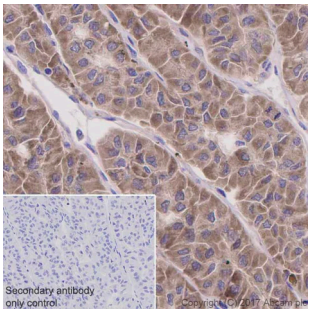
8 product images



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

ATG9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ATG9A antibody

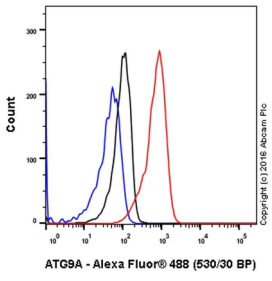
Unpurified ab108338, at 1/100, staining ATG9A in paraffin-embedded Human colon tissue by Immunohistochemistry.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108338).



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

ATG9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ATG9A antibody

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).



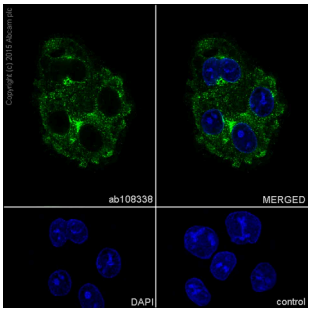
Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] - BSA and Azide free (ab223528)

ATG9A Flow Cytometry (Intracellular) staining using rabbit Anti-ATG9A antibody

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)

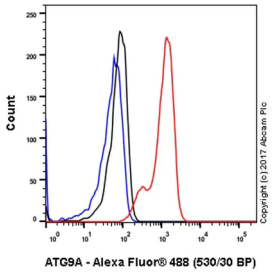
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108338).



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

ATG9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG9A antibody

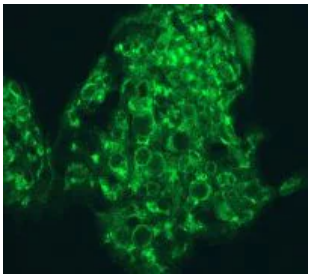
Immunocytochemistry/ Immunofluorescence analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with Purified ab108338 at 1:100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. ab150077 Goat anti rabbit IgG (Alexa Fluor® 488) was 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).



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

ATG9A Flow Cytometry (Intracellular) staining using rabbit Anti-ATG9A antibody

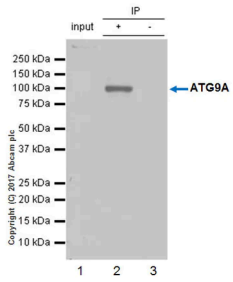
Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with purified ab108338 at 1:20 dilution (10 µg/ml) (red). Cells were fixed with 100% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used 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).



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

ATG9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG9A antibody

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).



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

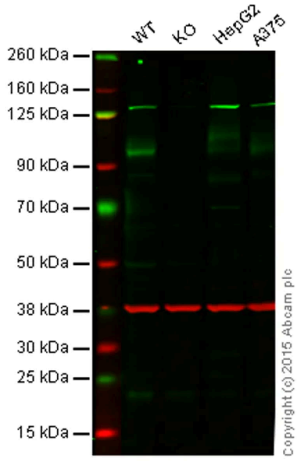
ab108338 (purified) at 1:20 dilution (2µg) immunoprecipitating ATG9A in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate.

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 secondary antibody (HRP) (ab131366) was used as the secondary antibody 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).

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

Predicted band size: 94 kDa



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

ATG9A Western blot staining using rabbit Anti-ATG9A antibody

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.

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

Predicted band size: 94 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.