abcam

ab249146

Anti-ATG4B antibody [EPR6436(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	EPR6436(2)
Purification technique	Affinity purification Protein A
Concentration	1.003 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-

Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Expected

Species	Mouse, Human
Dilution info	Use at an assay dependent concentration.

Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information ATG4B

Function	Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins (PubMed:15169837, PubMed:15187094, PubMed:17347651, PubMed:19322194, PubMed:21177865, PubMed:22302004, PubMed:26378241, PubMed:27527864, PubMed:28633005, PubMed:28821708, PubMed:29232556, PubMed:30076329, PubMed:30443548, PubMed:30661429). Required for canonical autophagy (macroautophagy), non-canonical autophagy as well as for mitophagy (PubMed:33773106, PubMed:33909989). The protease activity is required for proteolytic activation of ATG8 family proteins: cleaves the C-terminal amino acid of ATG8 proteins MAP1LC3A, MAP1LC3B, MAP1LC3C, GABARAPL1, GABARAPL2 and GABARAP, to reveal a C-terminal glycine (PubMed:15169837, PubMed:15187094, PubMed:17347651, PubMed:19322194, PubMed:20818167, PubMed:21177865, PubMed:22302004, PubMed:27527864, PubMed:28287329, PubMed:28633005, PubMed:29458288, PubMed:30661429). Exposure of the glycine at the C-terminus is essential for ATG8 proteins conjugation to phosphatidylethanolamine (PE) and insertion to membranes, which is necessary for autophagy (PubMed:15169837, PubMed:15187094, PubMed:17347651, PubMed:19322194, PubMed:21177865, PubMed:22302004). Protease activity is also required to counteract formation of high-molecular weight conjugates of ATG8 proteins (ATG8ylation): acts as a deubiquitinating-like enzyme that removes ATG8 conjugated to other proteins, such as ATG3 (PubMed:31315929, PubMed:33773106). In addition to the protease activity, also mediates delipidation of ATG8 family proteins (PubMed:15187094, PubMed:19322194, PubMed:28633005, PubMed:29458288, PubMed:32686895, PubMed:33909989). Catalyzes delipidation of PE-conjugated forms of ATG8 proteins during macroautophagy (PubMed:15187094, PubMed:19322194, PubMed:29458288, PubMed:32686895, PubMed:33909989). Also involved in non-canonical autophagy, a parallel pathway involving conjugation of ATG8 proteins to single membranes at endolysosomal compartments, by catalyzing delipidation of ATG8 proteins conjugated to phosphatidylserine (PS) (PubMed:33909989). Compared to other members of the family (ATG4A, ATG4C or ATG4C), constitutes the major protein for proteolytic activation of ATG8 proteins, while it displays weaker delipidation activity than other ATG4 paralogs (PubMed:29458288, PubMed:30661429). Involved in phagophore growth during mitophagy independently of its protease activity and of ATG8 proteins: acts by regulating ATG9A trafficking to mitochondria and promoting phagophore-endoplasmic reticulum contacts during the lipid transfer phase of mitophagy (PubMed:33773106).

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

ab249146 is the carrier-free version of ab154843.

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	ATG4B is a cysteine protease also known as autophagin-1 with a molecular mass of approximately 45 kDa. It is involved in the autophagy process by cleaving ATG8 family proteins. This protease facilitates the lipidation of LC3 an essential component of the autophagosome membrane. You find ATG4B expressed in various tissues especially in the brain liver and muscle where it plays important roles in cellular maintenance.
Biological function summary	ATG4B functions in the cellular autophagic machinery cleaving the C-terminal of ATG8 proteins. This processing is necessary for the conjugation to phosphatidylethanolamine and integration into autophagosomes. ATG4B is part of a larger autophagic complex that also includes other critical proteins like ATG7 and ATG3 helping prepare ATG8 proteins for autophagosome creation.
Pathways	ATG4B participates in the autophagy pathway by refining the cargo processing mechanism. It interacts with the mTOR signaling pathway which influences cell survival and growth based on nutrient availability. The ATG4B enzyme is closely related to ATG7 in the pathway as both contribute to the maturation of autophagosomes.

Associated diseases and disorders	ATG4B has significant links to cancer and neurodegenerative disorders. In cancer ATG4B activity affects tumor survival since cancer cells exploit autophagy for growth under stress conditions. In neurodegenerative diseases like Alzheimer's alterations in ATG4B activity disrupt cellular homeostasis. This disruption connects to proteins like LC3 which are pivotal in neuron survival and function.

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

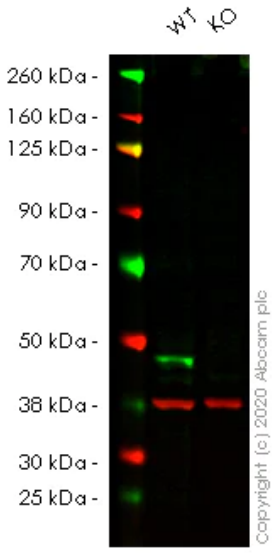
We do not recommend this combination. It is not covered by our product promise.

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.

3 product images



Western blot - Anti-ATG4B antibody [EPR6436(2)] - BSA and Azide free (ab249146)

ATG4B Western blot staining using rabbit Anti-ATG4B antibody

This data was developed using the same antibody clone in a different buffer formulation (ab154843).
Lanes 1 - 2: Merged signal (red and green). Green - ab154843 observed at 47 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab154843 was shown to react with ATG4B in wild-type HEK293T cells in Western blot with loss of signal observed in ATG4B knockout cell line ab265814 (ATG4B knockout cell lysate ab257364). Wild-type and ATG4B knockout HEK293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab154843 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

All lanes:
Western blot - Anti-ATG4B antibody [EPR6436(2)] (ab154843) at 1/1000 dilution

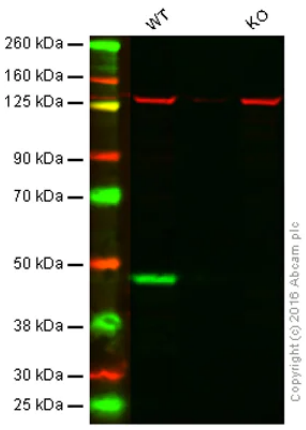
Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human ATG4B knockout HeLa cell lysate (ab257364) at 20 µg

Performed under reducing conditions.

Predicted band size: 44 kDa

Observed band size: 47 kDa



Western blot - Anti-ATG4B antibody [EPR6436(2)] - BSA and Azide free (ab249146)

ATG4B Western blot staining using rabbit Anti-ATG4B antibody

This data was developed using ab154843, the same antibody clone in a different buffer formulation.
Lane 1: Wild-type HAP1 cell lysate (20 µg)

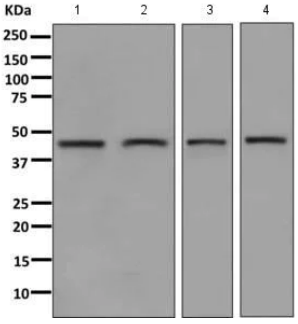
Lane 2: ATG4B knockout HAP1 cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - ab154843 observed at 47 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab154843 was shown to specifically react with ATG4B when ATG4B knockout samples were used. Wild-type and ATG4B knockout samples were subjected to SDS-PAGE. ab154843 and ab18058 (loading control to Vinculin) were diluted at 1/1000 and 1/10000 respectively 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 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-ATG4B antibody [EPR6436(2)] (ab154843)

Predicted band size: 44 kDa



Western blot - Anti-ATG4B antibody [EPR6436(2)] - BSA and Azide free (ab249146)

This data was developed using ab154843, the same antibody clone in a different buffer formulation.
All lanes:
Western blot - Anti-ATG4B antibody [EPR6436(2)] (ab154843) at 1/1000 dilution

Lane 1:
Ramos cell lysate at 10 µg

Lane 2:
293T cell lysate at 10 µg

Lane 3:
Jurkat cell lysate at 10 µg

Lane 4:
HeLa cell lysate at 10 µg

Predicted band size: 44 kDa

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