abcam

ab108251

Anti-ATG3 antibody [EPR4801]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR4801
Purification technique	Affinity purification Protein A
Concentration	0.101 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	Perform antigen retrieval before commencing with IHC staining protocol. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/10000 - 1/50000
Notes	-

Expected

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

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/2000
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information ATG3

Function

E2 conjugating enzyme that catalyzes the covalent conjugation of the C-terminal Gly of ATG8-like proteins (GABARAP, GABARAPL1, GABARAPL2 or MAP1LC3A) to the amino group of phosphatidylethanolamine (PE)-containing lipids in the membrane resulting in membrane-bound ATG8-like proteins which is one of the key steps in the development of autophagic isolation membranes during autophagosome formation (PubMed:24191030, PubMed:33446636, PubMed:37252361). Cycles back and forth between binding to ATG7 for loading with the ATG8-like proteins and binding to E3 enzyme, composed of ATG12, ATG5 and ATG16L1 to promote ATG8-like proteins lipidation (PubMed:11825910, PubMed:12207896, PubMed:12890687, PubMed:16704426, PubMed:24186333). Also plays a role as a membrane curvature sensor that facilitates LC3/GABARAP lipidation by sensing local membrane stress associated with lipid-packing defects as occurs with high molar proportions of conical lipids or strident membrane curvature (By similarity). Interacts with negatively-charged membranes promoting membrane tethering and enhancing LC3/GABARAP lipidation (PubMed:29142222). Also acts as an autocatalytic E2-like enzyme by catalyzing the conjugation of ATG12 to itself in an ATG7-dependent manner, this complex thus formed, plays a role in mitochondrial homeostasis but not in autophagy (By similarity). ATG12-ATG3 conjugation promotes late endosome to lysosome trafficking and basal autophagosome maturation via its interaction with PDCD6IP (By similarity). ATG12-ATG3 conjugate is also formed upon viccina

virus infection, leading to the disruption the cellular autophagy which is not necessary for vaccinia survival and proliferation (By similarity). Promotes primary ciliogenesis by removing OFD1 from centriolar satellites via the autophagic pathway (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

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.

Supplementary info

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

Activity summary	ATG3 also known as Autophagy-related protein 3 is an essential enzyme in the autophagy process. Its molecular weight is approximately 36 kDa. This protein facilitates the conjugation of LC3 (microtubule-associated protein 1A/1B-light chain 3) by acting as an E2-like enzyme. It is mainly expressed in tissues with high rates of autophagy including liver heart and brain.

Biological function summary	ATG3 plays an important role in the elongation of the autophagic membrane. It operates as a part of the autophagy machinery working closely with ATG7 an E1-like enzyme to transfer phosphatidylethanolamine (PE) to LC3 converting it into LC3-II. This lipidated form of LC3 is essential in the expansion and closure of the isolation membrane which forms the autophagosome the core structure for degradation and recycling of cellular components.
Pathways	ATG3 is a central player in the macroautophagy pathway a process critical for cellular homeostasis. It interacts with ATG7 to facilitate the lipidation of ATG8 family proteins which are important for autophagosome formation. Moreover ATG3 also links to apoptosis pathways where its balance with pro-apoptotic proteins helps regulate cell death processes.
Associated diseases and disorders	ATG3 has been implicated in neurodegenerative diseases like Alzheimer's. This relates partly to its role in protein clearance which is essential for preventing toxic aggregations. Altered ATG3 function can lead to accumulation of misfolded proteins exacerbating disease. Moreover ATG3's interaction with proteins in the cancer-related pathways such as p53 shows its involvement in tumorigenesis through its influence on cell survival and death mechanisms.

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

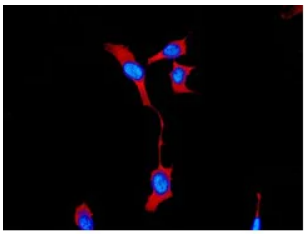
We do not recommend this combination. It is not covered by our product promise.

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.

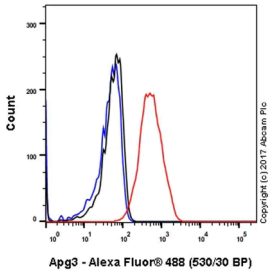
6 product images



Immunocytochemistry/ Immunofluorescence - Anti-ATG3 antibody [EPR4801] (ab108251)

ATG3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG3 antibody

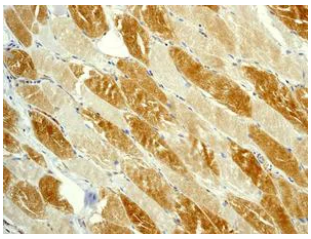
Immunofluorescent staining of HeLa cells using ab108251 at 1/100.



Flow Cytometry (Intracellular) - Anti-ATG3 antibody [EPR4801] (ab108251)

ATG3 Flow Cytometry (Intracellular) staining using rabbit Anti-ATG3 antibody

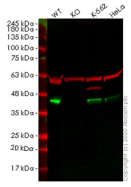
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling ATG3 (red) with ab108251 at a 1/2000 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (ab172730). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG3 antibody [EPR4801] (ab108251)

ATG3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ATG3 antibody

ab108251, at a 1/100 dilution, staining Human ATG3 in muscle, using Immunohistochemistry, Formalin/PFA-fixed paraffin-embedded tissue.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-ATG3 antibody [EPR4801] (ab108251)

ATG3 Western blot staining using rabbit Anti-ATG3 antibody

Lanes 1-4: Merged signal (red and green). Green - ab108251 observed at 40 kDa. Red - loading control ab7291 observed at 50 kDa.
ab108251 Anti-ATG3 antibody [EPR4801] was shown to specifically react with ATG3 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266707 (knockout cell lysate ab257363) was used. Wild-type and ATG3 knockout samples were subjected to SDS-PAGE.
ab108251 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-ATG3 antibody [EPR4801] (ab108251) at 1/1000 dilution

Lane 1:
Wild-type HEK293T cell lysate at 20 µg

Lane 2:
ATG3 knockout HEK293T cell lysate at 20 µg

Lane 2:
Western blot - Human ATG3 knockout HEK-293T cell line (ab266707)

Lane 3:
K-562 cell lysate at 20 µg

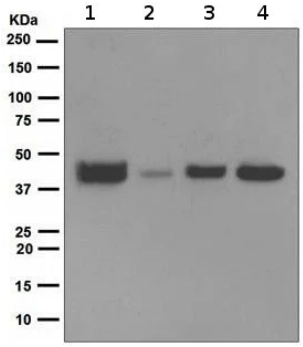
Lane 4:
HeLa cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 35 kDa

Observed band size: 40 kDa



Western blot - Anti-ATG3 antibody [EPR4801] (ab108251)

All lanes:
Western blot - Anti-ATG3 antibody [EPR4801] (ab108251) at 1/10000 dilution

Lane 1:
K562 cell lysate at 10 µg

Lane 2:
HL-60 cell lysate at 10 µg

Lane 3:
HeLa cell lysate at 10 µg

Lane 4:
Jurkat cell lysate at 10 µg

Secondary

All lanes:
HRP labelled goat anti-rabbit

Predicted band size: 35 kDa

Observed band size: 40 kDa

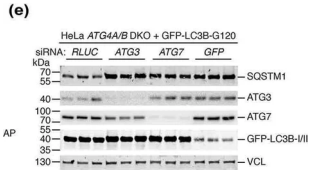


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Western blot - Anti-ATG3 antibody [EPR4801] (ab108251)

ATG3 western blot using anti-ATG3 antibody [EPR4801] ab108251. Publication image and figure legend from Agrotis, A., Pengo, N., et al., 2019, Autophagy, PubMed 30661429.

ab108251 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab108251 please see the product overview.

Additional loss of ATG4A in ATG4B KO cells only partially further reduces GABARAP subfamily priming and does not prevent rescue of autophagy by pre-primed LC3/GABARAP. (a) HeLa control, ATG4B KO and different ATG4A/B double knockout (DKO) clones were treated for 3 h with DMSO or

250 nM Torin1 + 10 nM baf A1 prior to lysis and western blot analysis. See also Figure S6A for analysis of the same clones by immunocytochemistry of endogenous GABARAPL1. (b) HeLa control, ATG4B KO and ATG4A/B DKO (c25) were transiently transfected with 3xFLAG-GABARAPL2-GFP. After 24 h, cells were treated for 3 h with DMSO or 250 nM Torin1 + 10 nM baf A1 prior to lysis and western blot. 3xFLAG-GABARAPL2-GFP was detected using anti-FLAG antibody. (c) HeLa cells stably transduced with GFP-GABARAPL1-G116 (Control and ATG4A/B DKO c25) and GFP-LC3B-G120 (ATG4A/B DKO c25) were treated for 3 h with DMSO or 250 nM Torin1 + 10 nM baf A1 prior to fixation and immunocytochemistry to reveal endogenous SQSTM1 and LAMP1 localization. Scale bar: 10 μm. See also Figure S6B for immunocytochemistry of untransduced cells. (d) Untransduced and stably-transduced HeLa control and ATG4A/B DKO (c25) cells were assessed for SQSTM1 protein level by western blot, 48 h after transfection with siRNA targeting RLUC as a negative control (lanes without +), or EGFP (lanes with +). GFP-LC3B-G120 and GFP-GABARAPL1-G116 were detected using anti-GFP antibody. (e) HeLa ATG4A/B DKO (c25) cells stably expressing GFP-LC3B-G120 were transfected with siRNA targeting RLUC (negative control), ATG3, ATG7 or GFP (positive control). After 48 h, cells were lysed and subject to western blot analysis to assess SQSTM1 protein level. GFP-LC3B-G120 was detected using anti-GFP antibody.

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