abcam

Product datasheet

Anti-APG5L/ATG5 antibody [EPR1755(2)] ab108327

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-APG5L/ATG5 antibody [EPR1755(2)]
Description	Rabbit monoclonal [EPR1755(2)] to APG5L/ATG5
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IP, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Raji, HeLa, HT-1080, Human fetal kidney, C6, Raw264.7, PC-12 and NIH3T3 cell lysates; Human hepatocellular carcinoma and Human ovarian adenocarcinoma tissue
General notes	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**.

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR1755(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108327 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (1)	1/100 - 1/250.
WB	★★★★☆ (4)	1/1000 - 1/10000. Predicted molecular weight: 32 kDa.
IP		1/10 - 1/100.
IHC-P	★★★★☆ (1)	1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Antigen retrieval is recommended.

Target

Function

Involved in autophagic vesicle formation. Conjugation with ATG12, through a ubiquitin-like conjugating system involving ATG7 as an E1-like activating enzyme and ATG10 as an E2-like conjugating enzyme, is essential for its function. The ATG12-ATG5 conjugate acts as an E3-like enzyme which is required for lipidation of ATG8 family proteins and their association to the vesicle membranes. Involved in mitochondrial quality control after oxidative damage, and in subsequent cellular longevity. The ATG12-ATG5 conjugate also negatively regulates the innate antiviral immune response by blocking the type I IFN production pathway through direct association with RARRES3 and MAVS. Also plays a role in translation or delivery of incoming viral RNA to the translation apparatus. Plays a critical role in multiple aspects of lymphocyte development and is essential for both B and T lymphocyte survival and proliferation. Required for optimal processing and presentation of antigens for MHC II. Involved in the maintenance of axon morphology and membrane structures, as well as in normal adipocyte differentiation. Promotes primary ciliogenesis through removal of OFD1 from centriolar satellites and degradation of IFT20 via the autophagic pathway.
May play an important role in the apoptotic process, possibly within the modified cytoskeleton. Its expression is a relatively late event in the apoptotic process, occurring downstream of caspase activity. Plays a crucial role in IFN-gamma-induced autophagic cell death by interacting with FADD.

Tissue specificity

Ubiquitous. The mRNA is present at similar levels in viable and apoptotic cells, whereas the protein is dramatically highly expressed in apoptotic cells.

Sequence similarities

Belongs to the ATG5 family.

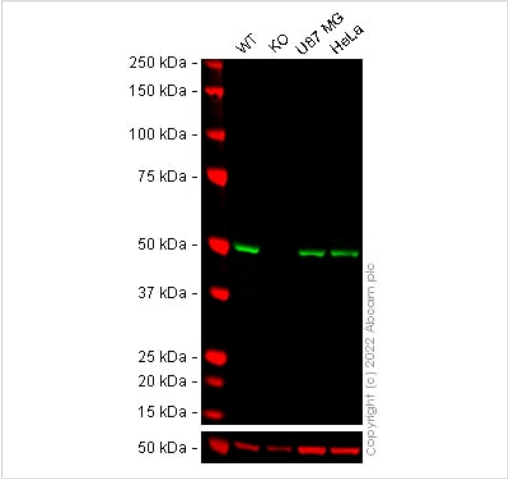
Post-translational modifications

Conjugated to ATG12; which is essential for autophagy, but is not required for association with isolation membrane.
Acetylated by EP300.

Cellular localization

Cytoplasm. Preautophagosomal structure membrane. Colocalizes with nonmuscle actin. The conjugate detaches from the membrane immediately before or after autophagosome formation is completed (By similarity). Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme.

Images



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

All lanes : Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327) at 1/1000 dilution

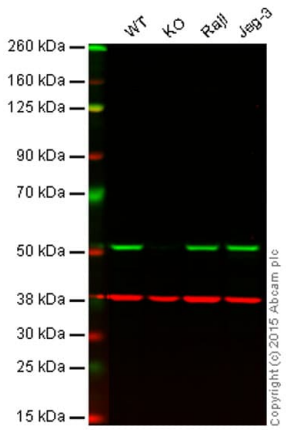
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ATG5 knockout THP-1 cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 32 kDa
Observed band size: 50 kDa

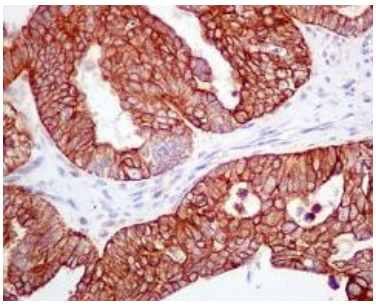
False colour image of Western blot: Anti-APG5L/ATG5 antibody [EPR1755(2)] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab108327 was shown to bind specifically to APG5L/ATG5. A band was observed at 50 kDa in wild-type THP-1 cell lysates with no signal observed at this size in ATG5 knockout cell line **ab277835** (knockout cell lysate **ab290722**). To generate this image, wild-type and ATG5 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

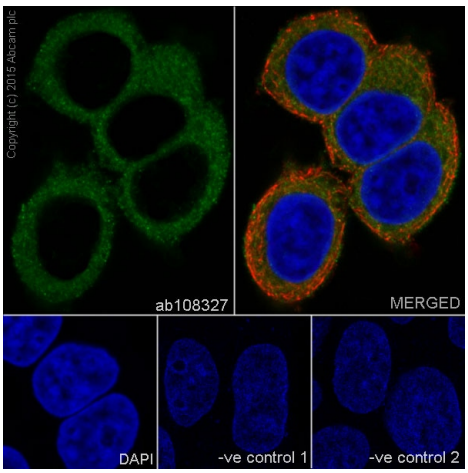
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: APG5L/ATG5 knockout HAP1 cell lysate (20 µg)
Lane 3: Raji cell lysate (20 µg)
Lane 4: Jeg-3 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab108327 observed at 52 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab108327 was shown to specifically react with APG5L/ATG5 when APG5L/ATG5 knockout samples were used. Wild-type and APG5L/ATG5 knockout samples were subjected to SDS-PAGE. ab108327 and **ab8245** (loading control to GAPDH) were both diluted 1/1000 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 h at room temperature before imaging.



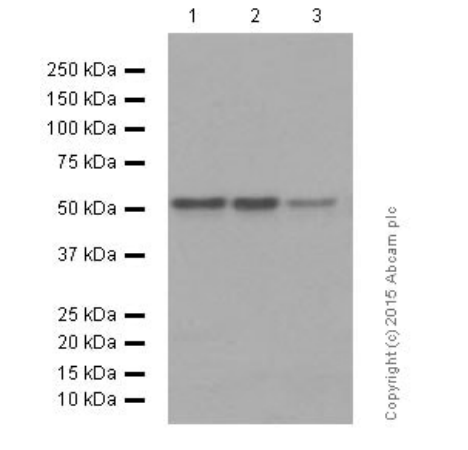
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Unpurified ab108327, at 1/100 dilution, staining APG5L/ATG5 in paraffin-embedded Human ovarian adenocarcinoma tissue by Immunohistochemistry.



Immunocytochemistry/ Immunofluorescence - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Immunofluorescence staining of MCF7 cells with purified ab108327 at a working dilution of 1/150, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 100% methanol and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab108327 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

All lanes : Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327) at 1/10000 dilution (purified)

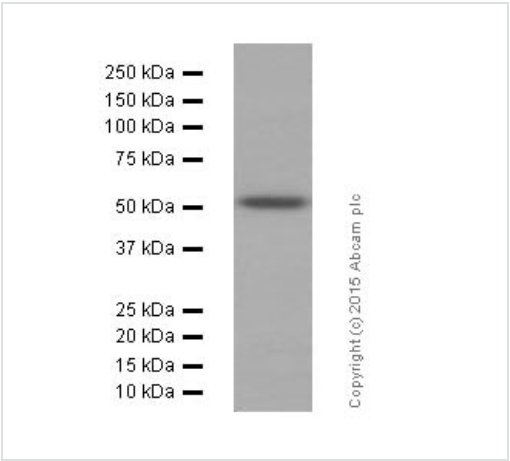
Lane 1 : C6 cell lysate
Lane 2 : PC-12 cell lysate
Lane 3 : NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 32 kDa
Observed band size: 55 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327) at 1/10000 dilution (purified) + Raji cell lysate at 20 µg

Secondary

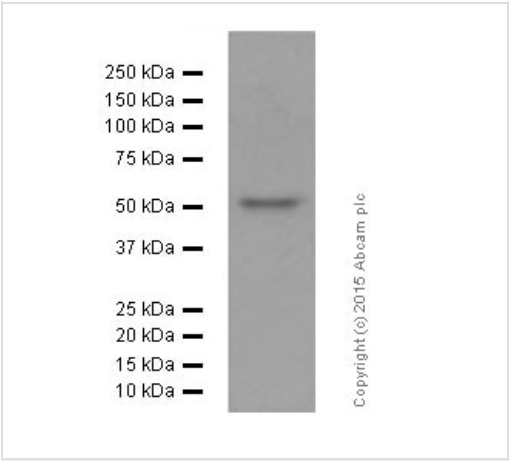
HRP goat anti-rabbit IgG (H+L) at 1/10000 dilution

Predicted band size: 32 kDa

Observed band size: 55 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327) at 1/2000 dilution (purified) + HT-1080 cell lysate at 20 µg

Secondary

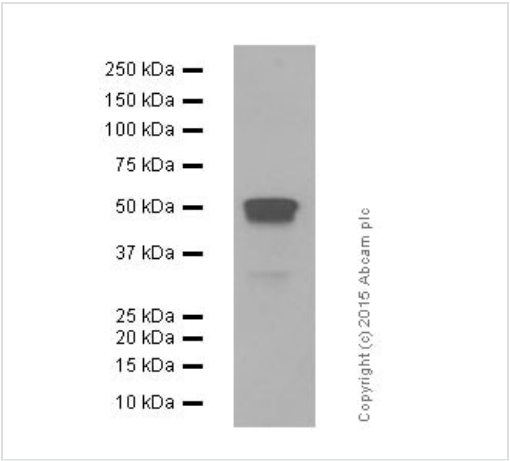
HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 32 kDa

Observed band size: 55 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327) at 1/2000 dilution (purified) + human fetal kidney at 10 µg

Secondary

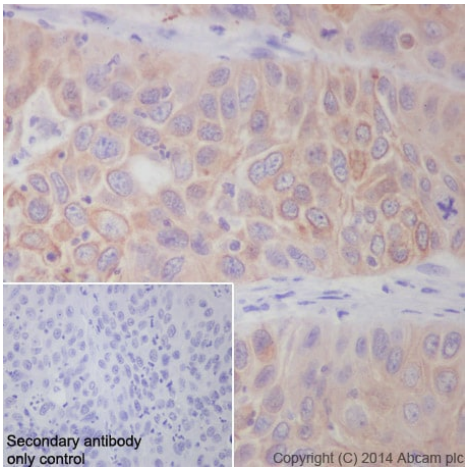
HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 32 kDa

Observed band size: 32,55 kDa

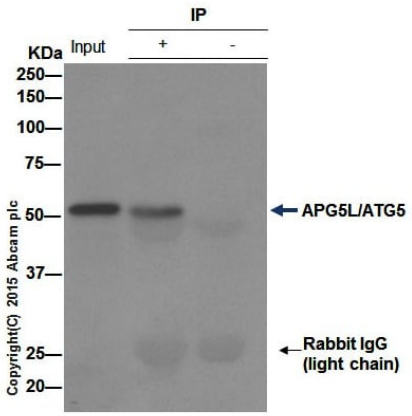
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



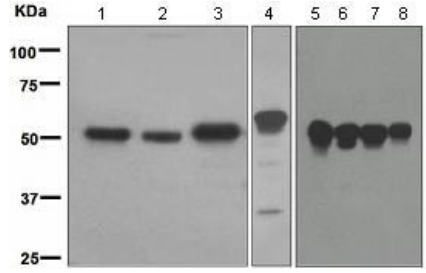
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified **ab180327** at a working dilution of 1/150. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunoprecipitation - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

ab180327 (purified) at 1/20 immunoprecipitating CRSP8 in 10 µg PC-12 whole cell lysate (Lanes 1 and 2, observed at 55 kDa). Lane 3 - PBS. For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1500 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



Western blot - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

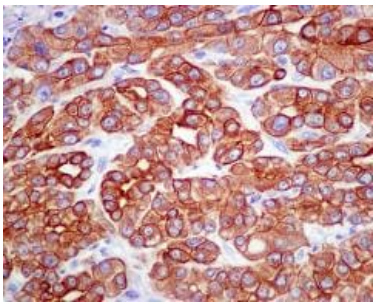
All lanes : Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327) at 1/1000 dilution (unpurified)

Lane 1 : Raji cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : HT-1080 cell lysate
Lane 4 : Human fetal kidney cell lysate
Lane 5 : C6 cell lysate
Lane 6 : Raw264.7 cell lysate
Lane 7 : PC-12 cell lysate
Lane 8 : NIH3T3 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 32 kDa

Secondary antibody - **anti-rabbit HRP** (**ab6721**)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

Unpurified ab108327, at 1/100 dilution, staining APG5L/ATG5 in paraffin-embedded Human hepatocellular carcinoma tissue by Immunohistochemistry.

Anti-APG5L/ATG5 antibody [EPR1755(2)] (ab108327)

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