abcam

Product datasheet

Anti-APG5L/ATG5 antibody [EPR4797] ab109490

KO VALIDATED Recombinant RabMAb

37 References 8 Images

Overview

Product name	Anti-APG5L/ATG5 antibody [EPR4797]
Description	Rabbit monoclonal [EPR4797] to APG5L/ATG5
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	THP1, Raji, HeLa, HT1080 and Human fetal kidney lysates; Human breast carcinoma and Human kidney tissues.
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.1% BSA, 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Tissue culture supernatant
Clonality	Monoclonal

Clone number	EPR4797
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109490 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
Flow Cyt (Intra)		1/250. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/10000. Predicted molecular weight: 32 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Application notes Is unsuitable for IP.

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.

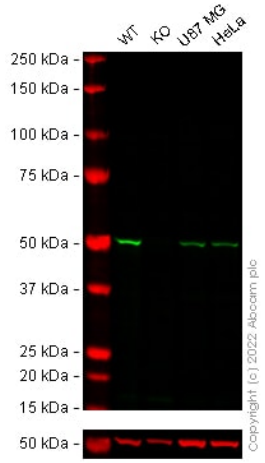
Sequence similarities Belongs to the ATG5 family.

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 [EPR4797] (ab109490)

All lanes : Anti-APG5L/ATG5 antibody [EPR4797] (ab109490) at 1/1000 dilution

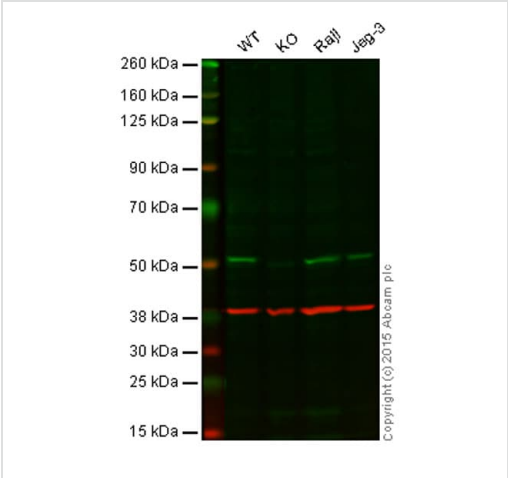
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ATG5 knockout THP-1 cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

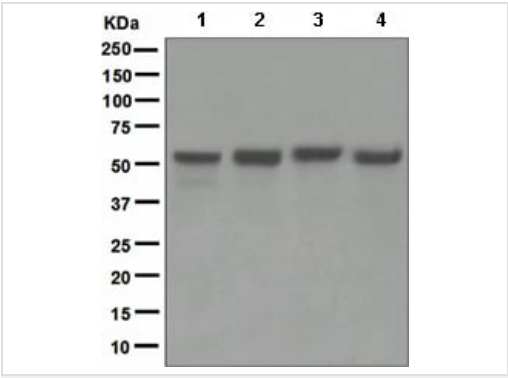
Predicted band size: 32 kDa
Observed band size: 50 kDa

False colour image of Western blot: Anti-APG5L/ATG5 antibody [EPR4797] 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, ab109490 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 [EPR4797] (ab109490)

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 - ab109490 observed at 52 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab109490 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. ab109490 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/2000 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 1/10 000 dilution for 1 h at room temperature before imaging.



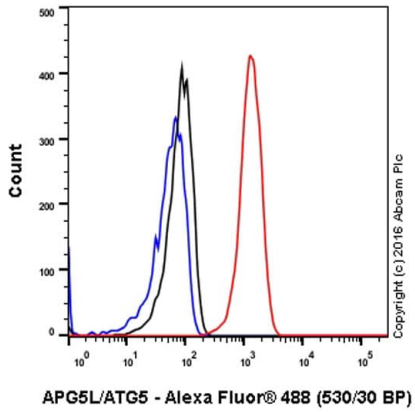
Western blot - Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

All lanes : Anti-APG5L/ATG5 antibody [EPR4797] (ab109490) at 1/1000 dilution

Lane 1 : THP1 cell lysate
Lane 2 : Raji cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : HT1080 cell lysate

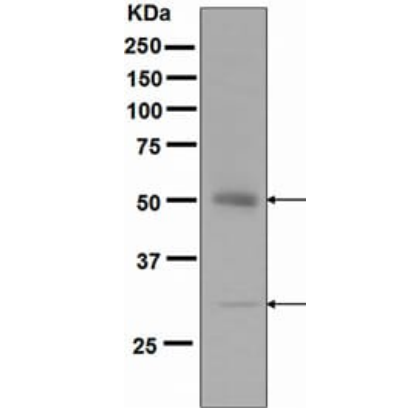
Lysates/proteins at 10 µg per lane.

Predicted band size: 32 kDa



Flow Cytometry (Intracellular) - Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling APG5L/ATG5 with purified ab109490 at 1/250 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr®488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

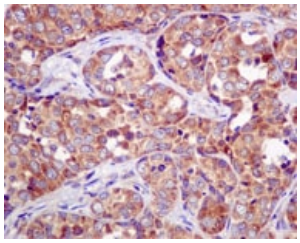


Western blot - Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

Anti-APG5L/ATG5 antibody [EPR4797] (ab109490) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

Predicted band size: 32 kDa

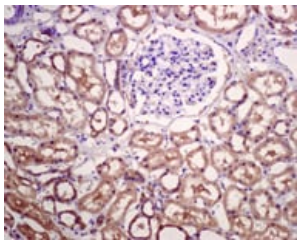
Upper band shows APG5L/ATG5 conjugated to ATG12.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

ab109490, at 1/100 dilution, staining APG5L/ATG5 in paraffin-embedded Human breast carcinoma by Immunohistochemistry.

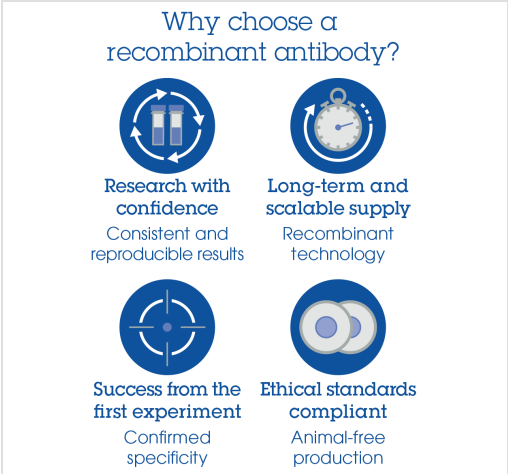
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

ab109490, at 1/100 dilution, staining APG5L/ATG5 in paraffin-embedded Human kidney by Immunohistochemistry.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

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