abcam

Product datasheet

Anti-ATG16L1 (phospho S278) antibody [EPR19016] ab195242

Recombinant RabMAb

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Overview

Product name	Anti-ATG16L1 (phospho S278) antibody [EPR19016]
Description	Rabbit monoclonal [EPR19016] to ATG16L1 (phospho S278)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, Dot blot, WB
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293 overexpressing ATG16L1 (WT) whole cell lysate. IHC-P: Human muscle tissue.
General notes	Co-immunization performed with both peptides, clone obtained by screening with peptide 1. 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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19016
Isotype	IgG

Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab195242 in the following tested applications.

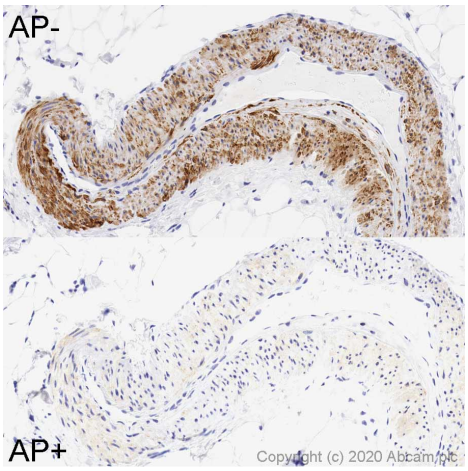
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/150.
IHC-P		Use a concentration of 3 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Dot blot		1/1000.
WB	★★★★★ **(5)**	Use at an assay dependent concentration. Predicted molecular weight: 68 kDa. For optimal WB signal, we recommend using 10X Blocking Buffer (**ab126587**).

Target

Function	Plays an essential role in autophagy: interacts with ATG12-ATG5 to mediate the conjugation of phosphatidylethanolamine (PE) to LC3 (MAP1LC3A, MAP1LC3B or MAP1LC3C), to produce a membrane-bound activated form of LC3 named LC3-II. Thereby, controls the elongation of the nascent autophagosomal membrane.
Involvement in disease	Inflammatory bowel disease 10
Sequence similarities	Belongs to the WD repeat ATG16 family. Contains 7 WD repeats.
Post-translational modifications	Proteolytic cleavage by activated CASP3 leads to degradation and may regulate autophagy upon cellular stress and apoptotic stimuli.
Cellular localization	Cytoplasm. Preautophagosomal structure membrane. Recruited to omegasomes membranes by WIPI2. Omegasomes are endoplasmic reticulum connected strutures at the origin of preautophagosomal structures. Localized to preautophagosomal structure (PAS) where it is involved in the membrane targeting of ATG5. Localizes also to discrete punctae along the ciliary axoneme.
Form	There are 4 isoforms produced by alternative splicing.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

IHC images of vessel staining of ab195242, ATG16L1 (phospho S278), in sections of formalin-fixed paraffin-embedded normal human skeletal muscle tissue*, performed on a Leica BOND™ system using a modified protocol F.

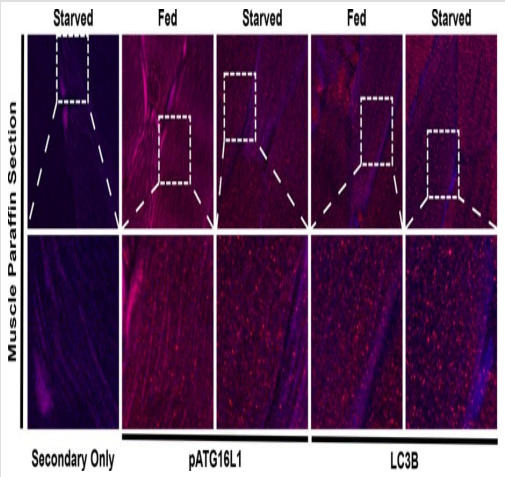
Both sections were pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. One section was then treated with 200 enzyme units of alkaline phosphatase (AP+) for 1 hour at 37°C; and the other in buffer containing no alkaline phosphatase (AP-) for 1 hour at 37°C. The sections were then incubated with ab195242, 3µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The sections were then counterstained with haematoxylin and mounted with DPX.

Identical assays were also performed using detection system-only (no primary antibody) as reagent controls (data not shown), to ensure that staining seen was a result of the binding of the primary antibody.

The absence of staining in the AP+ tissue compared to the AP- tissue adds further evidence of phospho specificity for this antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

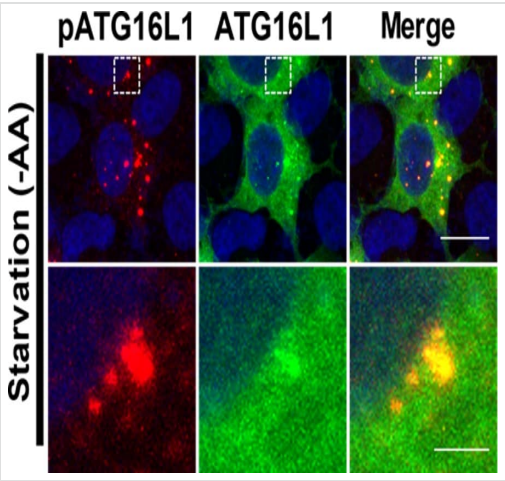


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

This image is courtesy of Dr. Ryan Russell (University of Ottawa).

IHC images of mice quadricep showing either pATG16L1 or LC3B staining

Mice were fed ad libitum or starved for 16 hours. Quadricep muscle were immediately harvested and fixed in 10% formalin for 2 days. The samples were then paraffin embedded, sectioned into 4µm thick slices, and mounted onto glass microscope slides. Slides were stained with primary antibody overnight at 4˚C: LC3B 1/1000, pATG16L1 (ab195242) 1/300. Secondary antibody: Alexa Fluor 555 anti-rabbit, 1/1000.

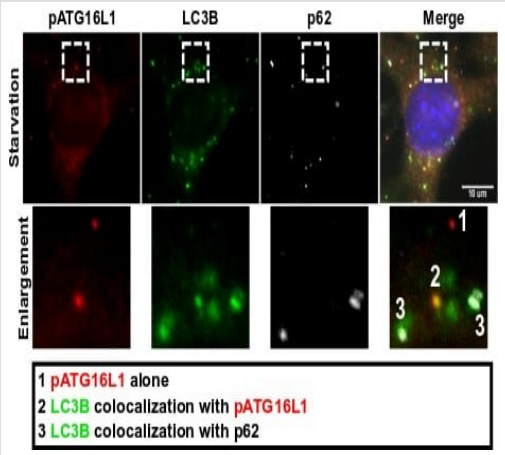


Immunocytochemistry/ Immunofluorescence - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

This image is courtesy of Dr. Ryan Russell (University of Ottawa).

IF showing pATG16L1 (red) and total ATG16L1 (green):

Polyclonal population of ATG16L1 KO and HA-ATG16L1 reconstituted cells were starved of amino acid for 1 hour and stained. Blocking buffer used for pATG16L1 staining: 0.1% BSA, 1x abcam blocking buffer **ab126587**, diluted in PBS. Anti-pATG16L1 (ab195242) concentration: 1/150. Anti-ATG16L1, concentration: 1/200 Secondary antibody (Alexa Fluor 647/488) concentration: 1/1000.

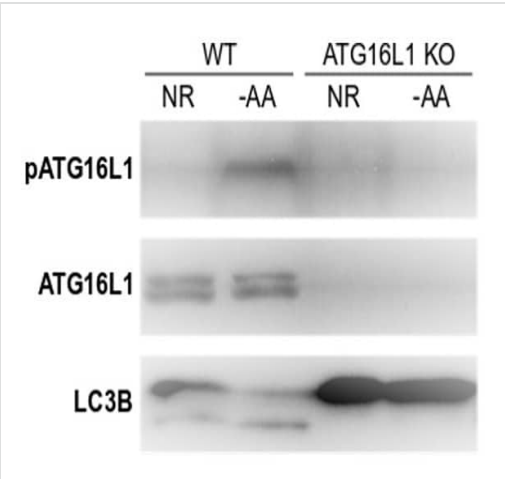


Immunocytochemistry/ Immunofluorescence - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

This image is courtesy of Dr. Ryan Russell (University of Ottawa).

IF showing pATG16L1 (red), LC3B (green) and p62 (white):

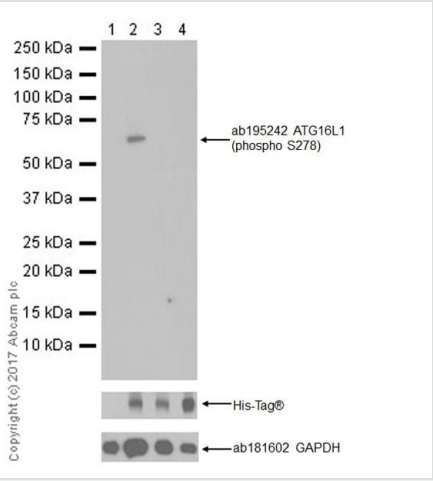
MEF cells were amino acid starved for 1 hour. Blocking buffer used for pATG16L1 (ab195242): 0.1% BSA, 1x abcam blocking buffer (**ab126587**), diluted in PBS. Anti-pATG16L1 (ab195242) concentration: 1/150. Secondary antibody (Alexa Fluor 647) concentration: 1/1000



Western blot - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

This image is courtesy of Dr Ryan Russell (University of Ottawa).

HCT116 wild-type and ATG16L1 knockout cells were incubated with either complete media or amino acid deficient DMEM for 3 hours. 5ug of whole cell lysate were resolved by SDS-PAGE on a 6%-18% gradient gel, then transferred onto PVDF membrane. Membrane was blocked in 10X blocking buffer (Cat # **ab126587**) diluted in TBS solution for 30 minutes; incubated with 1:1000 primary antibody in 2.5% BSA TBST solution overnight at 4°C ; incubated with 1:15000 secondary antibody in 2% milk TBST solution for 45 minutes. Immobilon ECL was applied for 1 minute then imaged with film.



Western blot - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

All lanes : Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242) at 1/1000 dilution

Lane 1 : HEK-293 (human epithelial cell line from embryonic kidney) transfected with an empty vector (vector control), containing a myc-His-tag®, whole cell lysate
Lane 2 : HEK-293 (human epithelial cell line from embryonic kidney) transfected with ATG16L1 (WT) expression vector containing a myc-His-tag®, whole cell lysate
Lane 3 : HEK-293 (human epithelial cell line from embryonic kidney) transfected with ATG16L1 (WT) expression vector containing a myc-His-tag®, followed by treatment with alkaline phosphatase for 1 hour, whole cell lysate
Lane 4 : HEK-293 (human epithelial cell line from embryonic kidney) transfected with ATG16L1 S278A expression vector containing a myc-His-tag®, whole cell lysate

Lysates/proteins at 10 µg per lane.

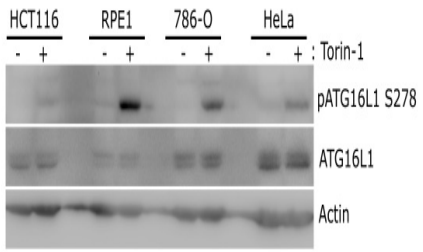
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 68 kDa
Observed band size: 68 kDa

Exposure time: 3 minutes

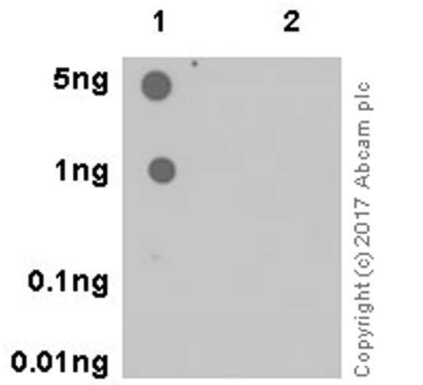
Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

This image is courtesy of Dr Ryan Russell (University of Ottawa).

Cells were treated with Torin-1 (200uM) for 1 hour. Membrane was blocked in 10X blocking buffer (Cat # **ab126587**) diluted in PBS solution for 30 minutes; incubated with 1/1000 primary antibody in 2.5% BSA TBST solution overnight at 4°C ; incubated with 1/7000 secondary antibody in 2% milk TBST solution for 1 hour.



Dot Blot - Anti-ATG16L1 (phospho S278) antibody [EPR19016] (ab195242)

Dot blot analysis of ATG16L1 (phospho S278) labeled with ab195242 at 1/1,000 dilution.

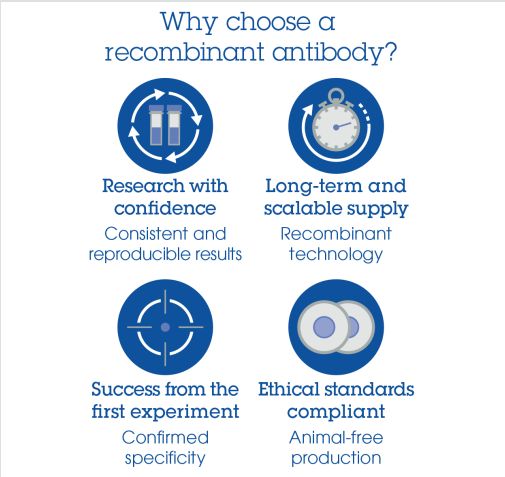
Lane 1: Mouse ATG16L1 (phospho S278) peptide;

Lane 2: Mouse ATG16L1 non-phospho peptide;

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100,000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Anti-ATG16L1 (phospho S278) antibody [EPR19016]

(ab195242)

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