

Product datasheet

Autophagy Analysis (ATG16L1, ATG16L1 pS278, SQSTM1, LC3B, Ubiquitin, M6PR) Antibody Sampler Panel ab269811

Recombinant

13 Images

Overview

Product name	Autophagy Analysis (ATG16L1, ATG16L1 pS278, SQSTM1, LC3B, Ubiquitin, M6PR) Antibody Sampler Panel
Species reactivity	Reacts with: Mouse, Rat, Human
Product overview	<p>Please note that Recombinant Anti-ATG16L1 (phospho S278) antibody [EPR19016] is for mouse and human reactivity only. The rest of the clones in this panel react with human, mouse, and rat.</p> <p>Autophagy Analysis (ATG16L1, ATG16L1 pS278, SQSTM1, LC3B, Ubiquitin, M6PR) Antibody Sampler Panel ab269811 contains multiple trial-sized versions of anti-human, mouse and rat antibody clones against ATG16L1, ATG16L1 pS278, SQSTM1, LC3B, Ubiquitin and M6PR, specifically selected for high performance in various applications. This panel contains 6 recombinant rabbit monoclonal antibodies against human, mouse and rat ATG16L1, ATG16L1 pS278, SQSTM1, LC3B, Ubiquitin and M6PR. They are provided as a sampler panel to allow you to easily evaluate each antibody.</p> <p>For guidelines on how to use each antibody within the panel, please consult the individual datasheet for each antibody.</p> <p>Panel contains:</p> <p>Recombinant Anti-ATG16L1 (phospho S278) antibody [EPR19016] (20 µL) ab195242</p> <p>Recombinant Anti-ATG16L1 antibody [EPR15638] - N-terminal antibody (20 µL) ab187671</p> <p>Recombinant Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker antibody (20 µL) ab109012</p> <p>Recombinant Anti-LC3B antibody [EPR18709] - Autophagosome Marker antibody (20 µL) ab192890</p>

Recombinant Anti-Ubiquitin antibody [EPR8830] (20 µL) [ab134953](#)

Recombinant Anti-M6PR (cation independent) antibody [EPR6599] - Lysosome Membrane Marker (20 µL) [ab124767](#)

Please note that Recombinant Anti-ATG16L1 (phospho S278) antibody [EPR19016] is for mouse reactivity only. The rest of the clones in this panel react with human, mouse, and rat.

For optimal WB results using [ab195242](#), we recommend blocking with 10X Blocking Buffer ([ab126587](#)).

Notes

Explore our range of antibody sample panels designed to provide you with a variety of trial-size antibodies in a convenient and cost-effective format.

Directly conjugated versions of our antibodies are available and ready to use for multicolor flow cytometry or immunocytochemistry analysis. Please refer to the 'Associated products' section below.

Carrier-free formulations of our recombinant antibodies are also available for easy conjugation to labels of your choice and for multiplex applications. Please refer to the 'Associated products' section below.

Tested applications

Suitable for: IHC-P, WB, ICC/IF

Properties

Storage instructions Store at -20°C. Please refer to protocols.

Components	1 kit
ab195242 - Recombinant Anti-ATG16L1 (phospho S278) antibody [EPR19016]	2 x 10µl
ab187671 - Recombinant Anti-ATG16L1 antibody [EPR15638] - N-terminal	2 x 10µl
ab192890 - Recombinant Anti-LC3B antibody [EPR18709] - Autophagosome Marker	2 x 10µl
ab124767 - Recombinant Anti-M6PR (cation independent) antibody [EPR6599] - Lysosome Membrane Marker	2 x 10µl
ab109012 - Recombinant Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker	2 x 10µl
ab134953 - Recombinant Anti-Ubiquitin antibody [EPR8830]	2 x 10µl

Cellular localization

Ubiquitin: Cell Membrane, Cytoplasmic and Nuclear M6PR (cation independent): Lysosome membrane. Colocalized with DPP4 in internalized cytoplasmic vesicles adjacent to the cell surface. SQSTM1 / p62: Cytoplasm. Late endosome. Nucleus. Sarcomere (By similarity). In cardiac muscles localizes to the sarcomeric band (By similarity). Localizes to late endosomes. May also localize to the nucleus. Accumulates in neurofibrillary tangles and in Lewy bodies of neurons from individuals with Alzheimer and Parkinson disease respectively. Enriched in Rosenthal fibers of pilocytic astrocytoma. In liver cells, accumulates in Mallory bodies associated with alcoholic hepatitis, Wilson disease, indian childhood cirrhosis and in hyaline bodies associated with hepatocellular carcinoma. ATG16L1: Cytoplasm. Preautophagosomal structure

membrane. Recruited to omegasomes membranes by WIPI2. Omegasomes are endoplasmic reticulum connected structures 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. LC3B: Cytoplasm > cytoskeleton. Endomembrane system. Cytoplasmic vesicle > autophagosome membrane. LC3-II binds to the autophagic membranes.

Applications

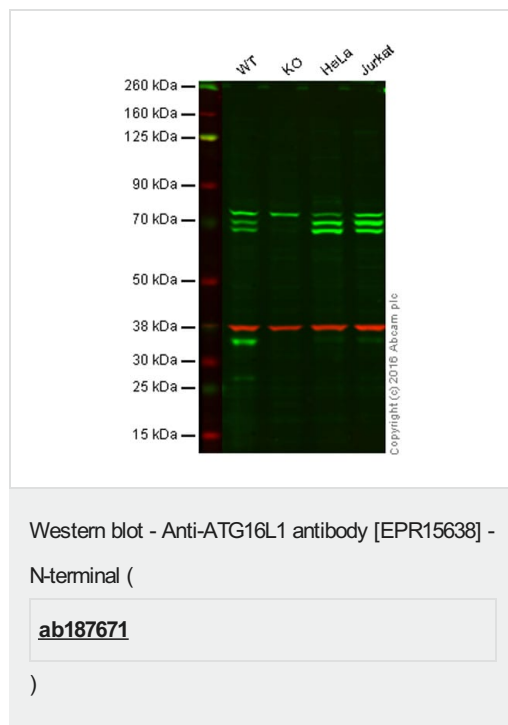
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab269811 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
IHC-P		Use at an assay dependent concentration. ab109012 has not been in house tested for IHC-P.
WB		Use at an assay dependent concentration. For optimal WB results using ab195242 , we recommend blocking with 10X Blocking Buffer (ab126587)
ICC/IF		Use at an assay dependent concentration. ab187671 has not been in house tested for ICC/IF.

Images



Lane 1: Wild-type HAP1 cell lysate (20 µg)

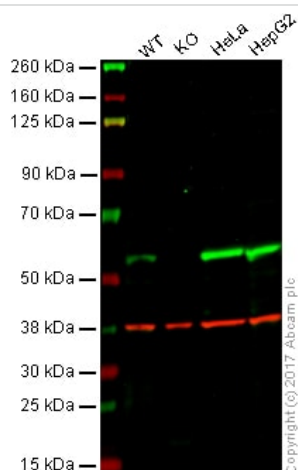
Lane 2: ATG16L1 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: Jurkat cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - Anti-ATG16L1 antibody [EPR15638] - N-terminal (**ab187671**) observed at 68 and 70 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab187671 was shown to recognize ATG16L1 when ATG16L1 knockout samples were used, along with additional cross-reactive bands. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. **ab187671** and **ab8245** (loading control to GAPDH) were diluted 1/2000 and 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 h at room temperature before imaging.



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (

ab109012

)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

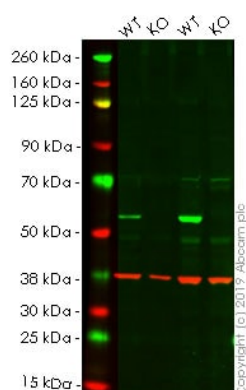
Lane 2: SQSTM1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: HepG2 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - Anti-SQSTM1 / p62 antibody [EPR4844] (**ab109012**) observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab109012 was shown to specifically react with SQSTM1 in wild-type HAP1 cells. No band was observed when SQSTM1 knockout samples were used. Wild-type and SQSTM1 knockout samples were subjected to SDS-PAGE, Ab109012 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/20,000 dilution respectively. Blots were developed 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 1/20,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (

ab109012

)

Lane 1: Hap1 wildtype cell lysate (20 µg)

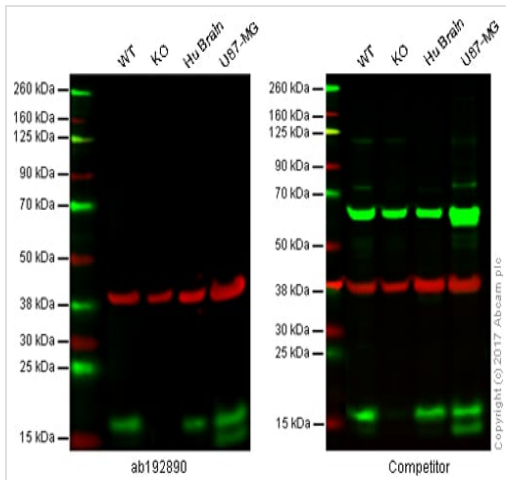
Lane 2: SQSTM1 Hap1 knockout cell lysate (20 µg)

Lane 3: HeLa wildtype cell lysate (20 µg)

Lane 4: SQSTM1 HeLa knockout cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - Anti-SQSTM1 / p62 antibody [EPR4844] (**ab109012**) observed at 64 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab109012 was shown to react with SQSTM1 / p62 in HeLa wildtype. Loss of signal was observed when knockout sample **ab263770** was used. Wild-type and SQSTM1 / p62 knockout samples were subjected to SDS-PAGE. **ab109012** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 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.



Western blot - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (

ab192890

)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: LC3B knockout HAP1 cell lysate (20 µg)

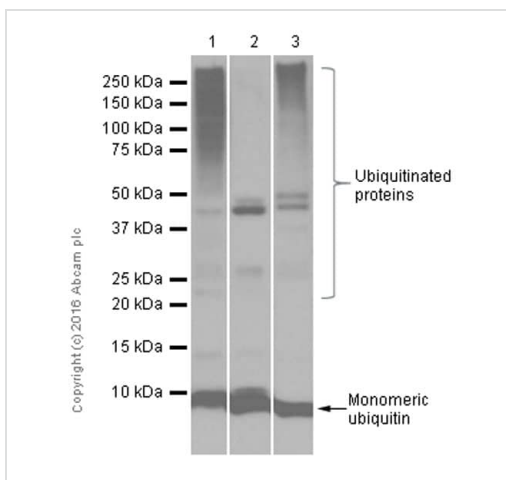
Lane 3: Human brain tissue lysate (20 µg)

Lane 4: U-87 MG cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green).

Green -target observed at 14 and 16 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between Anti-LC3B antibody [EPR18709] (**ab192890**) and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-Ubiquitin antibody [EPR8830] (

ab134953

)

Primary: Anti-Ubiquitin antibody [EPR8830] (**ab134953**), Purified, 0.7 µg/mL

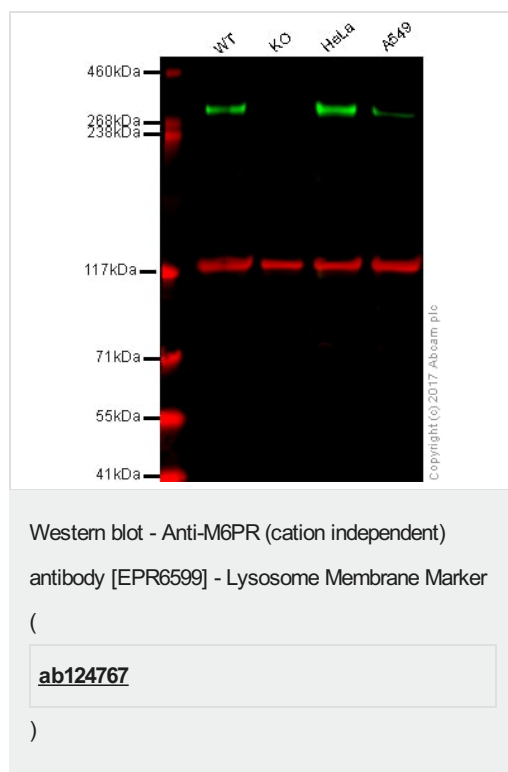
Lane 1: Rat brain lysate, 20 µg

Lane 2: Mouse kidney lysate, 20 µg

Lane 3: Rat kidney lysate, 20 µg

Secondary: **ab97051**, 1/20000 dilution

Blocking and diluting buffer: 5% NFDm/TBST.



Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

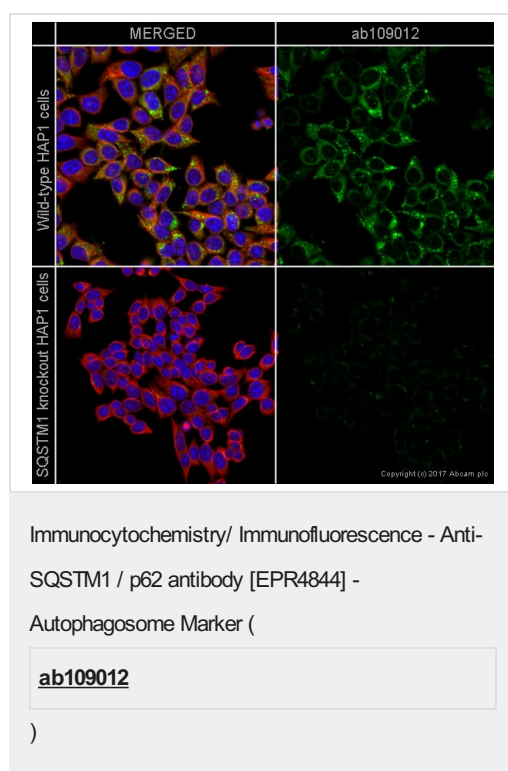
Lane 2: M6PR (cation independent) knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: A549 whole cell lysate (20 µg)

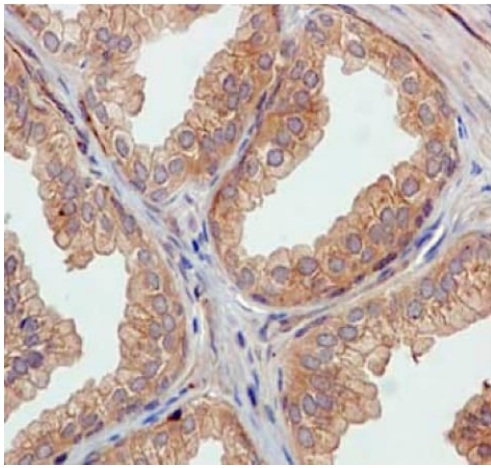
Lanes 1 - 4: Merged signal (red and green). Green - Anti-M6PR (cation independent) antibody [EPR6599] (**ab124767**) observed at 274 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab124767 was shown to specifically react with M6PR (cation independent) in wild-type HAP1 cells as signal was lost in M6PR (cation independent) knockout cells. Wild-type and M6PR (cation independent) knockout samples were subjected to SDS-PAGE. Ab124767 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/50000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/10000 dilution for 1 hour at room temperature before imaging.



Purified **ab109012** staining SQSTM1 in wild-type HAP1 cells (top panel) and SQSTM1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab109012** at 1 µg/ml and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



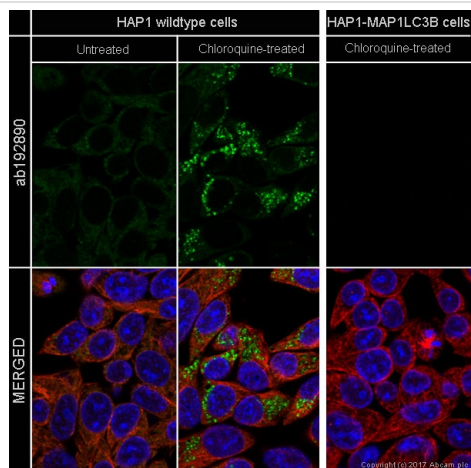
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG16L1 antibody [EPR15638] - N-terminal (

ab187671

)

Immunohistochemical analysis of paraffin-embedded Human prostatic hyperplasia tissue labeling ATG16L1 with **ab187671** at 1/100 dilution followed by pre-diluted HRP Polymer for Rabbit IgG secondary antibody and counter-stained with Hematoxylin.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (

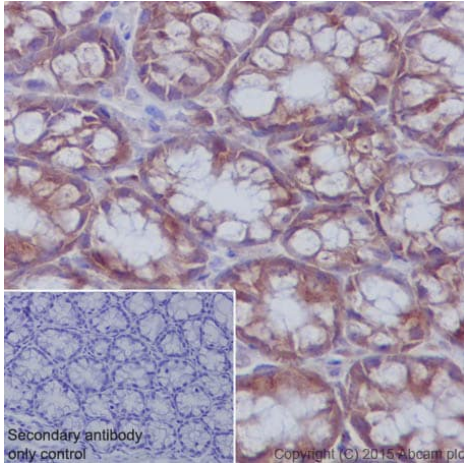
ab192890

)

ab192890 staining LC3B in HAP1 cells (wildtype and MAP1LC3B knockout) +/- Chloroquine (50µM, 24 hours).

The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab192890** at 1 µg/ml and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red) followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labeled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

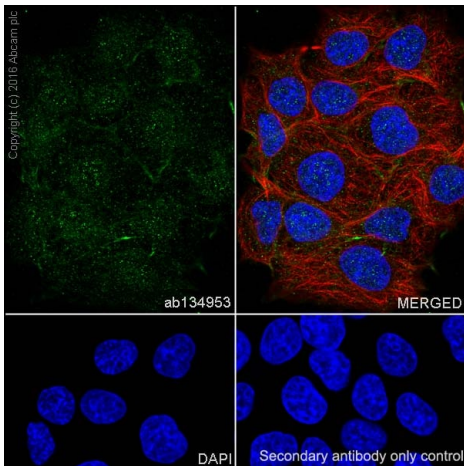


Immunohistochemical staining of paraffin embedded mouse colon tissue section labelling M6PR with purified **ab124767** at dilution of 1/500. The secondary antibody used was **ab97051** Goat Anti-Rabbit IgG H&L (HRP), at a dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed 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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-M6PR (cation independent) antibody [EPR6599] - Lysosome Membrane Marker (

ab124767

)

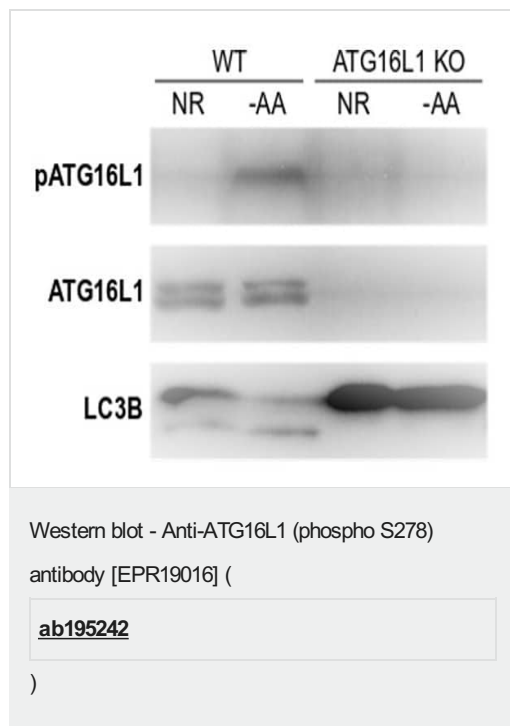


Immunocytochemistry/ Immunofluorescence analysis of JAR (Human placenta choriocarcinoma cell line) cells labeling Ubiquitin with Purified **ab134953** at 1:100 dilution (7.2µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin antibody [EPR8830] (

ab134953

)



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 Anti-ATG16L1 (phospho S278) antibody [EPR19016] ([ab195242](#)) at 1/1000 dilution 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.

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Autophagy Analysis (ATG16L1, ATG16L1 pS278, SQSTM1, LC3B, Ubiquitin, M6PR) Antibody Sampler Panel (ab269811)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Promise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you

- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors