abcam

Product datasheet

Anti-ATG16L1 antibody [EPR15638] - N-terminal ab187671

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-ATG16L1 antibody [EPR15638] - N-terminal
Description	Rabbit monoclonal [EPR15638] to ATG16L1 - N-terminal
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Raji, Jurkat, Daudi, PC12 and NIH 3T3 cell lysates, Wild-type THP-1 cell lysate, Wild type HeLa cell lysate IHC-P: Human prostatic hyperplasia and Mouse colon 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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR15638

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab187671 in the following tested applications.

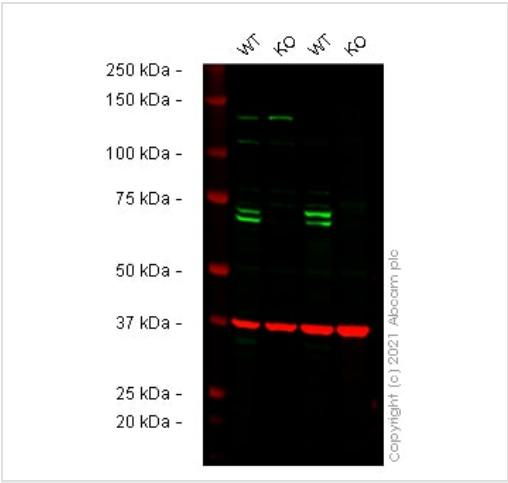
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (4)	1/1000 - 1/10000. Detects a band of approximately 68 kDa (predicted molecular weight: 68 kDa).
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/1000 dilution

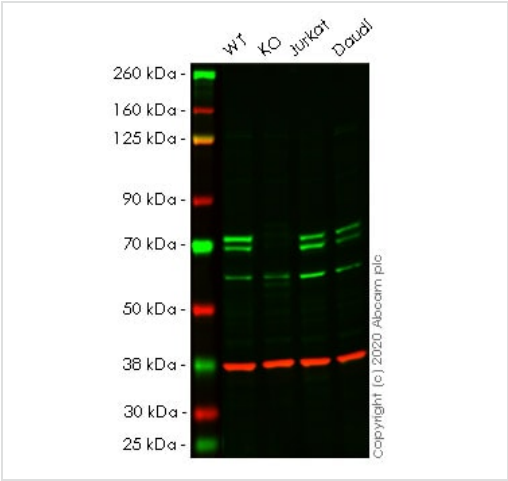
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ATG16L1 knockout THP-1 cell lysate
Lane 3 : Wild type HeLa cell lysate
Lane 4 : ATG16L1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 68,70 kDa

False colour image of Western blot: Anti-ATG16L1 antibody [EPR15638] - N-terminal staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab187671 was shown to bind specifically to ATG16L1. A band was observed at 68/70 kDa in wild-type THP-1 cell lysates with no signal observed at this size in ATG16L1 knockout cell line **ab277834** (knockout cell lysate **ab278184**). To generate this image, wild-type and ATG16L1 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 3 % 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG16L1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

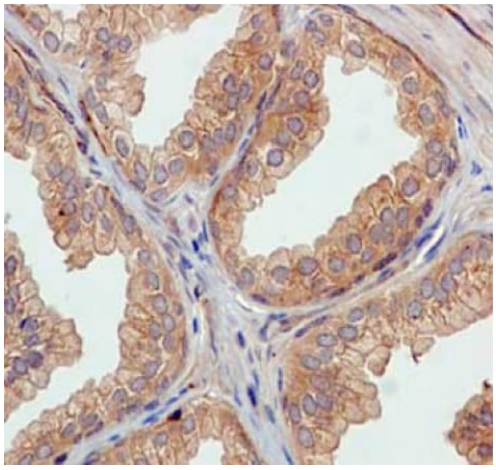
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1- 4: Merged signal (red and green). Green - ab187671 observed at 68 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

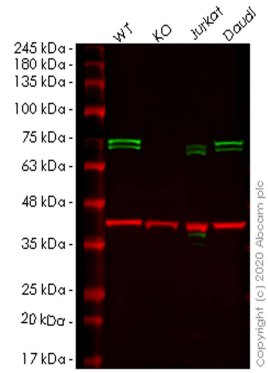
ab187671 was shown to react with ATG16L1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265263** (knockout cell lysate **ab256842**) was used. Wild-type HeLa and ATG16L1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab187671 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



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.



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG16L1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

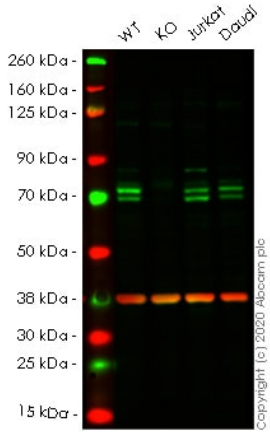
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 68 kDa
Observed band size: 75 kDa

Lanes 1-4: Merged signal (red and green). Green - ab187671 observed at 75 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab187671 Anti-ATG16L1 antibody [EPR15638] was shown to specifically react with ATG16L1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265263** (knockout cell lysate **ab256842**) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. ab187671 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours 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.



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG16L1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

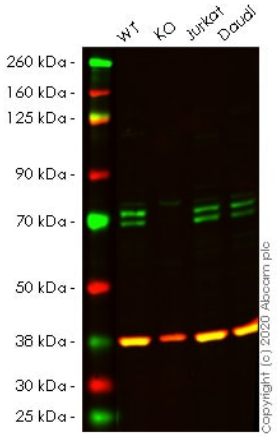
Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 68,72 kDa

Lanes 1-4: Merged signal (red and green). Green - ab187671 observed at 68 and 72 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab187671 Anti-ATG16L1 antibody [EPR15638] - N-terminal was shown to specifically react with ATG16L1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261773** (knockout cell lysate **ab256844**) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. ab187671 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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.



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG16L1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

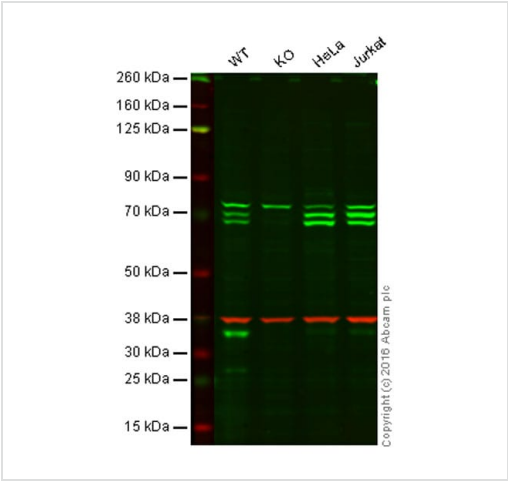
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 68,72 kDa

Lanes 1-4: Merged signal (red and green). Green - ab187671 observed at 68 and 72 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab187671 Anti-ATG16L1 antibody [EPR15638] - N-terminal was shown to specifically react with ATG16L1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261772** (knockout cell lysate **ab256843**) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. ab187671 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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.



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

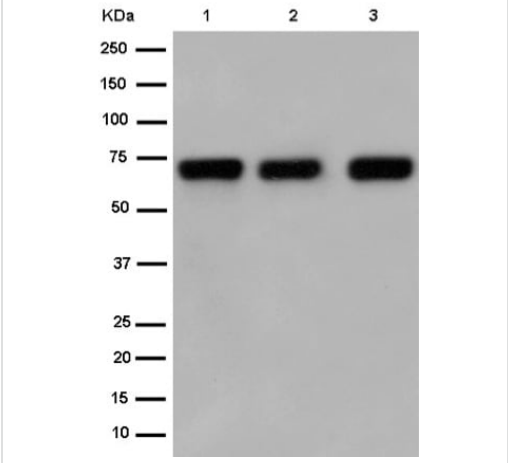
All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/2000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : ATG16L1 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa

Lanes 1 - 4: Merged signal (red and green). Green - 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-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

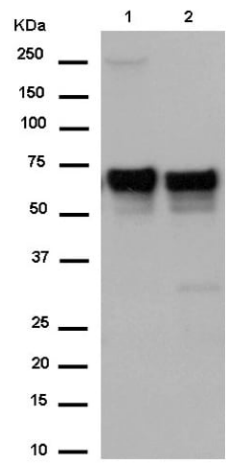
All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/5000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : Raji cell lysate
Lane 3 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 68 kDa



Western blot - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

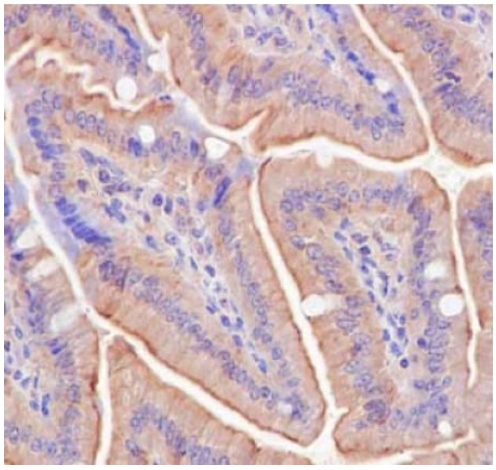
All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671) at 1/1000 dilution

Lane 1 : PC12 cell lysate
Lane 2 : NIH 3T3 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 68 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

Immunohistochemical analysis of paraffin-embedded Mouse colon 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.

Anti-ATG16L1 antibody [EPR15638] - N-terminal (ab187671)

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