

## Product datasheet

### Anti-ATG4B antibody [EPR16572] ab199537

KO VALIDATED Recombinant RabMAb

7 Images

#### Overview

Product name	Anti-ATG4B antibody [EPR16572]
Description	Rabbit monoclonal [EPR16572] to ATG4B
Host species	Rabbit
Tested applications	<b>Suitable for:</b> WB, IP
Species reactivity	<b>Reacts with:</b> Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: 293T, Ramos, HeLa, Jurkat and PC-12 whole cell lysates; Rat brain lysate. IP: Jurkat and Ramos whole cell lysates.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 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	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16572
Isotype	IgG

## Applications

### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab199537 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		1/1000. Detects a band of approximately 44 kDa (predicted molecular weight: 44 kDa).
IP		1/50.

## Target

### Function

Cysteine protease required for autophagy, which cleaves the C-terminal part of either MAP1LC3, GABARAPL2 or GABARAP, allowing the liberation of form I. A subpopulation of form I is subsequently converted to a smaller form (form II). Form II, with a revealed C-terminal glycine, is considered to be the phosphatidylethanolamine (PE)-conjugated form, and has the capacity for the binding to autophagosomes.

### Tissue specificity

Mainly expressed in the skeletal muscle, followed by brain, heart, liver and pancreas.

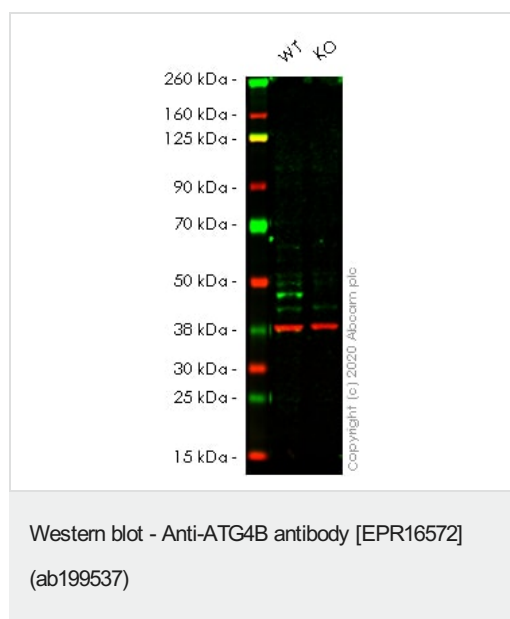
### Sequence similarities

Belongs to the peptidase C54 family.

### Cellular localization

Cytoplasm.

## Images



**All lanes :** Anti-ATG4B antibody [EPR16572] (ab199537) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** ATG4B knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

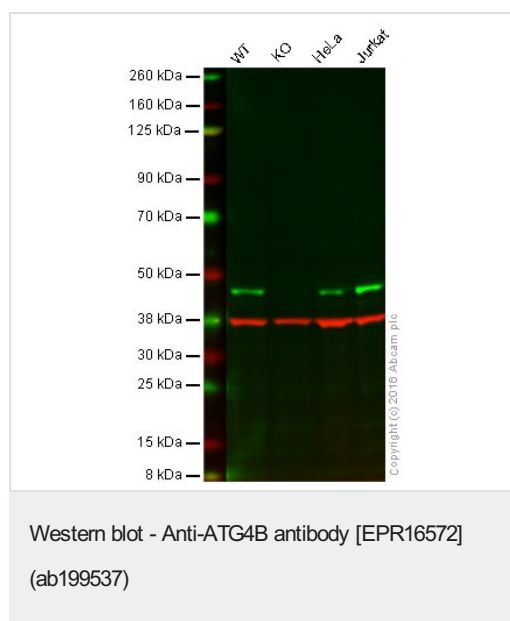
Performed under reducing conditions.

**Predicted band size:** 44 kDa

**Observed band size:** 47 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - ab199537 observed at 47 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab199537 was shown to react with ATG4B in wild-type HeLa cells in Western blot with loss of signal observed in ATG4B knockout cell line **ab265814** (ATG4B knockout cell lysate **ab257364**). Wild-type and ATG4B knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab199537 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 h at room temperature before imaging.



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

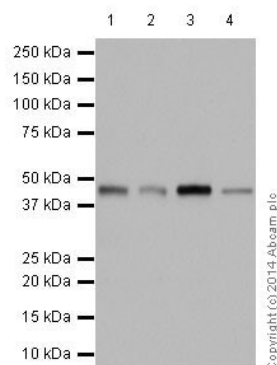
**Lane 2:** ATG4B knockout HAP1 cell lysate (20 µg)

**Lane 3:** HeLa cell lysate (20 µg)

**Lane 4:** Jurkat cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab199537 observed at 44 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab199537 was shown to specifically react with ATG4B when ATG4B knockout samples were used. Wild-type and ATG4B knockout samples were subjected to SDS-PAGE. ab199537 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10000 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 hour at room temperature before imaging.



Western blot - Anti-ATG4B antibody [EPR16572]  
(ab199537)

**All lanes :** Anti-ATG4B antibody [EPR16572] (ab199537) at  
1/1000 dilution

**Lane 1 :** 293T (Human epithelial cells from embryonic kidney)  
whole cell lysate

**Lane 2 :** Ramos (Human Burkitt's lymphoma cell line) whole cell  
lysate

**Lane 3 :** HeLa (Human epithelial cells from cervix  
adenocarcinoma) whole cell lysate

**Lane 4 :** Jurkat (Human T cell leukemia cells from peripheral blood)  
whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

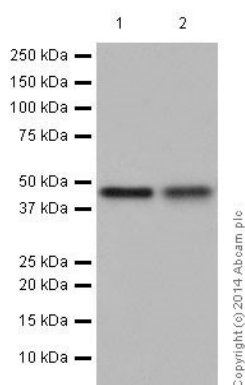
**All lanes :** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at  
1/1000 dilution

**Predicted band size:** 44 kDa

**Observed band size:** 44 kDa

**Exposure time:** 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ATG4B antibody [EPR16572]  
(ab199537)

**All lanes :** Anti-ATG4B antibody [EPR16572] (ab199537) at  
1/1000 dilution

**Lane 1 :** Rat brain tissue lysate

**Lane 2 :** PC-12 (Rat adrenal gland pheochromocytoma cell line)  
whole cell lysate

Lysates/proteins at 10 µg per lane.

### Secondary

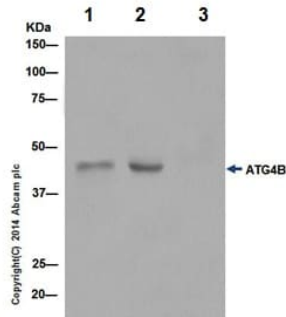
**All lanes :** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at  
1/1000 dilution

**Predicted band size:** 44 kDa

**Observed band size:** 44 kDa

**Exposure time:** 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



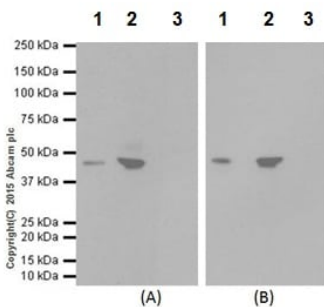
Immunoprecipitation - Anti-ATG4B antibody  
[EPR16572] (ab199537)

ATG4B was immunoprecipitated from 1mg of Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate with ab199537 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab199537 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: Jurkat whole cell lysate 10ug (Input). Lane 2: ab199537 IP in Jurkat whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab199537 in Jurkat whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



Immunoprecipitation - Anti-ATG4B antibody  
[EPR16572] (ab199537)

ATG4B was immunoprecipitated from 1mg of Ramos (Human Burkitt's lymphoma cell line) whole cell lysate with ab199537 at 1/60 dilution. Western blot was performed from the immunoprecipitate using ab199537 at 1/500 dilution (Panel A) or **ab154843** at 1/500 dilution (Panel B). Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: Ramos whole cell lysate 10ug (Input). Lane 2: ab199537 IP in Ramos whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab199537 in Ramos whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: Panel A and B 30 seconds.

### 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

Anti-ATG4B antibody [EPR16572] (ab199537)

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

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