abcam

# ab227084

# Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free

Recombinant | RabMAb | KO Validated

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Carrier free** | Yes |
| **Clone number** | EPR4797 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.993 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |

| | |
|---|---|
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Not recommended

| | |
|---|---|
| **Species** | Mouse, Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human, Mouse |
| **Dilution info** | - |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody. |

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information ATG5

**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. 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. As part of the ATG8 conjugation system with ATG12 and ATG16L1, required for recruitment of LRRK2 to stressed lysosomes and induction of LRRK2 kinase activity in response to lysosomal stress (By similarity). 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. (Microbial infection) May act as a proviral factor. In

association with ATG12, negatively regulates the innate antiviral immune response by impairing the type I IFN production pathway upon vesicular stomatitis virus (VSV) infection (PubMed:17709747). Required for the translation of incoming hepatitis C virus (HCV) RNA and, thereby, for initiation of HCV replication, but not required once infection is established (PubMed:19666601).

## Storage

| | |
|---|---|
| **Shipped at conditions** | Blue Ice |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | +4°C |
| **Storage information** | Do Not Freeze |

## Notes

ab227084 is the carrier-free version of ab109490.

**Conjugation ready**

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

**Compatibility**

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | APG5L commonly known as ATG5 or ATG5 protein plays a significant role in autophagy. This molecular component has a molecular weight of approximately 32 kDa. ATG5 is expressed in multiple tissue types with a higher expression profile in immune-related cells. The protein is essential for the formation of the autophagosome as it conjugates with ATG12 to form a complex that is critical for autophagosome membrane elongation. |
| **Biological function summary** | ATG5 plays an important part in the cellular process of autophagy affecting the degradation and recycling of cellular components. It is a part of a complex with ATG12 and ATG16L1 which facilitates the membrane building of the |

| | |
|---|---|
| | autophagosome. This protein is important for maintaining cellular homeostasis allowing the removal of damaged proteins and organelles and is involved in responses to stress and starvation. |
| **Pathways** | ATG5 is an important component in the autophagy pathway and is involved in cellular maintenance processes. This pathway is vital for the regulation of cell growth and metabolism. ATG5 works closely with other autophagy-related proteins such as LC3. The ATG5-ATG12 complex helps in the lipidation of LC3 which is critical for autophagosome maturation connecting its function to the mTOR signaling pathway that regulates many cellular processes. |
| **Associated diseases and disorders** | Disruption in ATG5 function is linked to neurodegenerative diseases and cancer. For instance dysregulation of autophagy due to ATG5 dysfunction may result in the progression of neurodegenerative disorders like Alzheimer's disease. Additionally in certain cancers altered autophagy can influence the survival of cancer cells. The interplay between ATG5 and other proteins like p53 and Bcl-2 is under investigation to understand its role in these diseases. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

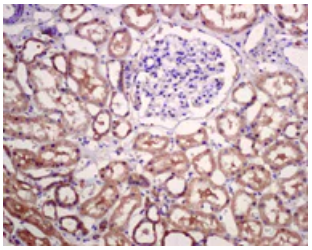
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

## 5 product images

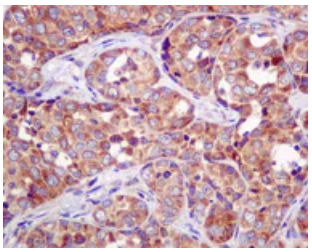


## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free (ab227084)

APG5L/ATG5 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-APG5L/ATG5 antibody

ab109490, at 1/100 dilution, staining APG5L/ATG5 in paraffin-embedded Human kidney by Immunohistochemistry.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109490).

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

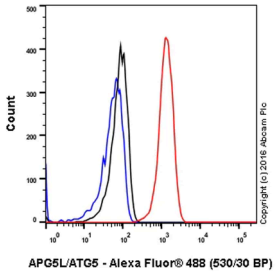


## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free (ab227084)

APG5L/ATG5 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-APG5L/ATG5 antibody

ab109490, at 1/100 dilution, staining APG5L/ATG5 in paraffin-embedded Human breast carcinoma by Immunohistochemistry.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109490).

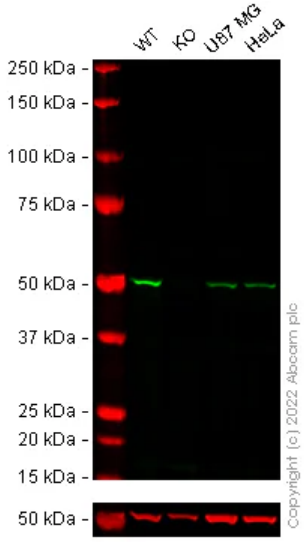
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.




## Flow Cytometry (Intracellular) - Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free (ab227084)

APG5L/ATG5 Flow Cytometry (Intracellular) staining using rabbit Anti-APG5L/ATG5 antibody

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 Fluor®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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109490).




## Western blot - Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free (ab227084)

APG5L/ATG5 Western blot staining using rabbit Anti-APG5L/ATG5 antibody

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

Lane 1:
Wild-type THP-1 cell lysate at 20 µg

Lane 2:
Western blot - Human ATG5 knockout THP-1 cell lysate (ab290722)

Lane 3:
U-87 MG cell lysate at 20 µg

Lane 4:
HeLa cell lysate at 20 µg

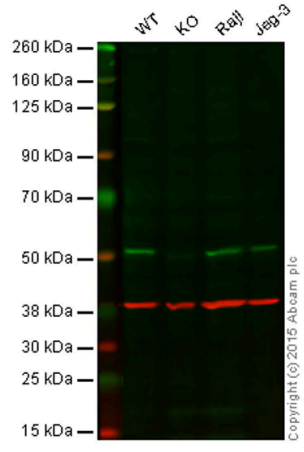
Secondary

Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 50 kDa




## Western blot - Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free (ab227084)

APG5L/ATG5 Western blot staining using rabbit Anti-APG5L/ATG5 antibody

This WB data was generated using the same anti-APG5/ATG5 antibody clone, EPR4797, in a different buffer formulation (cat# 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.
All lanes:
Western blot - Anti-APG5L/ATG5 antibody [EPR4797] (ab109490)

Predicted band size: 32 kDa

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Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.