abcam

Product datasheet

# Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free ab227084

敲除验证 重组 RabMAb

**4 References** 6 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-APG5L/ATG5抗体[EPR4797] - BSA and Azide free |
| **描述** | 兔单克隆抗体[EPR4797] to APG5L/ATG5 - BSA and Azide free |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** Flow Cyt (Intra), WB, IHC-P<br>**不适用于:** IP |
| **种属反应性** | **与反应:** Mouse, Human<br>**预测可用于:** Rat |
| **免疫原** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **阳性对照** | THP1, Raji, HeLa, HT1080 and Human fetal kidney lysates; Human breast carcinoma and Human kidney tissues. |
| **常规说明** | ab227084 is the carrier-free version of **ab109490**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>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.<br><br>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.<br><br>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. |

## 性能

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| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |

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|---|---|
| **存储溶液** | pH: 7.20<br>Constituent: PBS |
| **无载体** | **是** |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | EPR4797 |
| **同种型** | IgG |

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab227084于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|---|---|---|
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration.<br>**ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody. |
| **WB** | | Use at an assay dependent concentration. Predicted molecular weight: 32 kDa. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |

**应用说明** Is unsuitable for IP.

## 靶标

**功能** 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. The ATG12-ATG5 conjugate also negatively regulates the innate antiviral immune response by blocking the type I IFN production pathway through direct association with RARRES3 and MAVS. Also plays a role in translation or delivery of incoming viral RNA to the translation apparatus. 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.
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.

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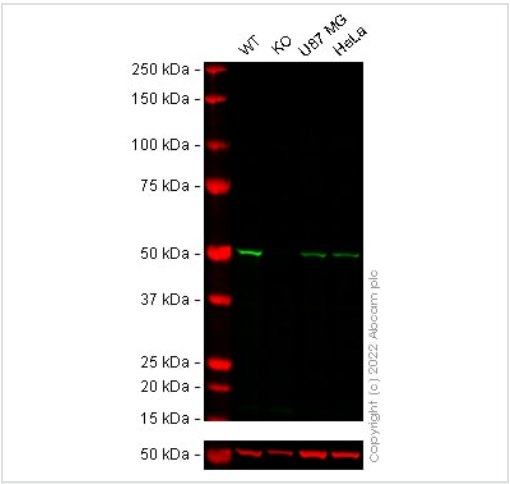
| **组织特异性** | Ubiquitous. The mRNA is present at similar levels in viable and apoptotic cells, whereas the protein is dramatically highly expressed in apoptotic cells. |
| **序列相似性** | Belongs to the ATG5 family. |
| **翻译后修饰** | Conjugated to ATG12; which is essential for autophagy, but is not required for association with isolation membrane.<br>Acetylated by EP300. |
| **细胞定位** | Cytoplasm. Preautophagosomal structure membrane. Colocalizes with nonmuscle actin. The conjugate detaches from the membrane immediately before or after autophagosome formation is completed (By similarity). Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme. |

## 图片




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

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

**Lane 1 :** Wild-type THP-1 cell lysate
**Lane 2 :** ATG5 knockout THP-1 cell lysate
**Lane 3 :** U-87 MG cell lysate
**Lane 4 :** HeLa cell lysate

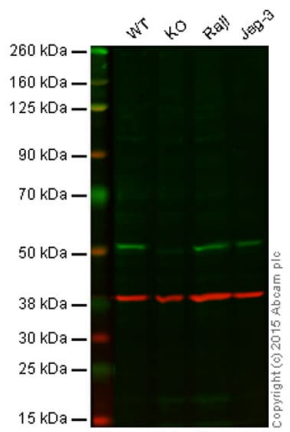
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 32 kDa
**Observed band size:** 50 kDa

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

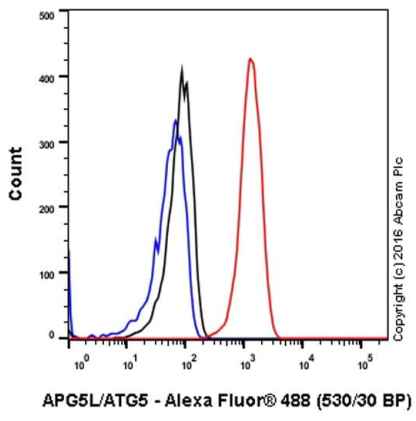
then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.




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

This WB data was generated using the same anti-APG5/ATG5 antibody clone, EPR4797, in a different buffer formulation (cat# **ab109490**).

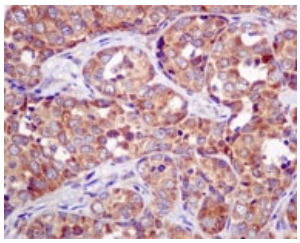
**Lane 1**: Wild-type HAP1 cell lysate (20 µg)
**Lane 2**: APG5L/ATG5 knockout HAP1 cell lysate (20 µg)
**Lane 3**: Raji cell lysate (20 µg)
**Lane 4**: Jeg-3 cell lysate (20 µg)
**Lanes 1 - 4**: Merged signal (red and green). Green - **ab109490** observed at 52 kDa. Red - loading control, **ab8245**, 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.




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

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**).

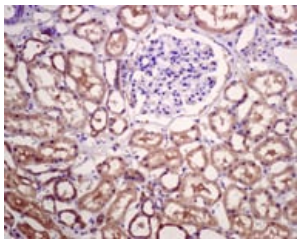


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APG5L/ATG5 antibody [EPR4797] - BSA and Azide free (ab227084)

**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.



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

**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.


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



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