# abcam

## Product datasheet

# Anti-ATG12 antibody [EPR4800] - BSA and Azide free ab247876



Recombinant

RabMAb

## 6 Images

#### Overview

Product name Anti-ATG12 antibody [EPR4800] - BSA and Azide free

**Description** Rabbit monoclonal [EPR4800] to ATG12 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB

Unsuitable for: Flow Cyt,ICC/IF or IP

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**General notes** ab247876 is the carrier-free version of <u>ab109491</u>.

Our <u>carrier-free</u> 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.

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.

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

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR4800

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab247876 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. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 15 kDa.

**Application notes** Is unsuitable for Flow Cyt,ICC/IF or IP.

**Target** 

**Function** Ubiquitin-like protein required for autophagy. Conjugated to ATG3 and ATG5.

Tissue specificity Ubiquitous.

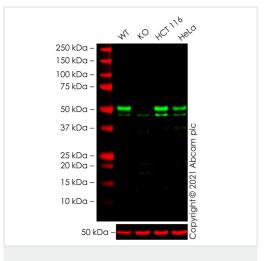
**Sequence similarities** Belongs to the ATG12 family.

**Domain** Shares weak sequence similarity with ubiquitin family, but contains an 'ubiquitin superfold' and the

C-terminal Gly is required for isopeptide linkage.

Cellular localization Cytoplasm.

#### **Images**



Western blot - Anti-ATG12 antibody [EPR4800] - BSA and Azide free (ab247876)

All lanes: Anti-ATG12 antibody [EPR4800] (ab109491) at 1/1000 dilution

Lane 1: Wild-type THP-1 cell lysate

Lane 2: ATG12 knockout THP-1 cell lysate

Lane 3: HCT 116 cell lysate

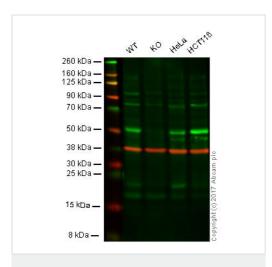
Lane 4: HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 52 kDa

False colour image of Western blot: Anti-ATG12 antibody [EPR4800] 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, ab109491 was shown to bind specifically to ATG12. A band likely to be the unfunctional complex with ATG5 was observed at 52 kDa in wild-type THP-1 cell lysates with no signal observed at this size in Atg12 knockout cell line ab277831 (knockout cell lysate ab278183) - unconjugated functional form not observed at 15 kDa. To generate this image, wild-type and Atg12 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 lgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-ATG12 antibody [EPR4800] - BSA and Azide free (ab247876)

This data was developed using <u>ab109491</u>, the same antibody clone in a different buffer formulation.

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

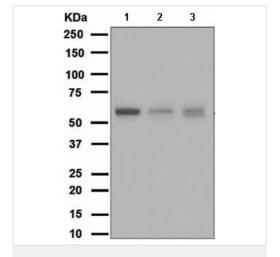
Lane 2: ATG12 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: HCT116 whole cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab109491</u> observed at 55 kDa. Observed band shows ATG12-ATG5 conjugation. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

Ab109491 was shown to specifically react with ATG12 in wild-type cells along with additional cross-reactive bands. The band was not seen in ATG12 knockout HAP1 cells. Wild-type and ATG12 knockout samples were subjected to SDS-PAGE. <a href="mailto:ab109491">ab109491</a> and <a href="mailto:ab245">ab245</a> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ATG12 antibody [EPR4800] - BSA and Azide free (ab247876)

All lanes: Anti-ATG12 antibody [EPR4800] (ab109491) at 1/1000 dilution

Lane 1: Human fetal kidney tissue lysate

Lane 2: HepG2 cell lysate

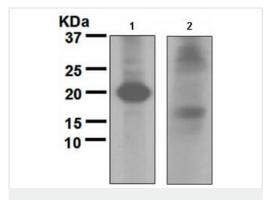
Lane 3: Raji lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 15 kDa

This data was developed using <u>ab109491</u>, the same antibody clone in a different buffer formulation.

Observed band shows ATG12-ATG5 conjugation.



Western blot - Anti-ATG12 antibody [EPR4800] - BSA and Azide free (ab247876)

**All lanes :** Anti-ATG12 antibody [EPR4800] (<u>ab109491</u>) at 1/1000 dilution

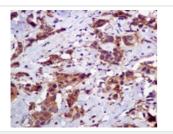
Lane 1: ATG12 transfected 293T lysate

Lane 2: Non-transfected 293T lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 15 kDa

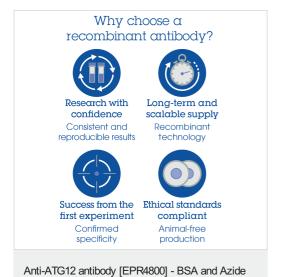
This data was developed using <u>ab109491</u>, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ATG12 antibody

[EPR4800] - BSA and Azide free (ab247876)

This data was developed using <u>ab109491</u>, the same antibody clone in a different buffer formulation.<u>ab109491</u>, at 1/250 dilution, staining ATG12 in paraffin-embedded Human breast carcinoma by Immunohistochemistry. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



free (ab247876)

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

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