


Product datasheet

Anti-ATG12 antibody [EPR4800] ab109491

KO VALIDATED Recombinant RabMAB[®]

[8 References](#) [6 Images](#)

Overview

Product name	Anti-ATG12 antibody [EPR4800]
Description	Rabbit monoclonal [EPR4800] to ATG12
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P 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.
Positive control	Human fetal kidney, HepG2, and Raji lysates; ATG12 transfected 293T lysates; Human breast carcinoma tissue.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR4800
Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109491 in the following tested applications.

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

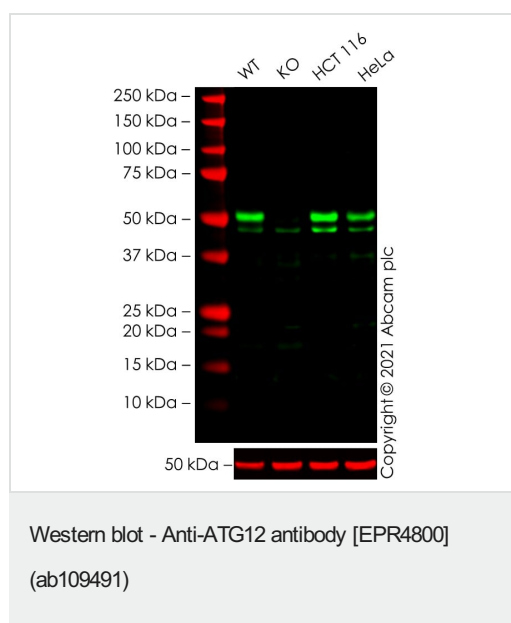
Application	Abreviews	Notes
WB		1/1000 - 1/10000. Predicted molecular weight: 15 kDa.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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



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

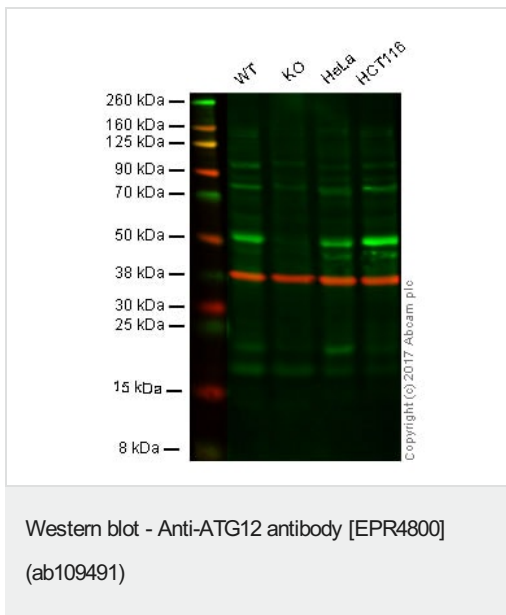
Lysates/proteins at 20 µg per lane.

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 IgG H&L (IRDye[®] 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



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)

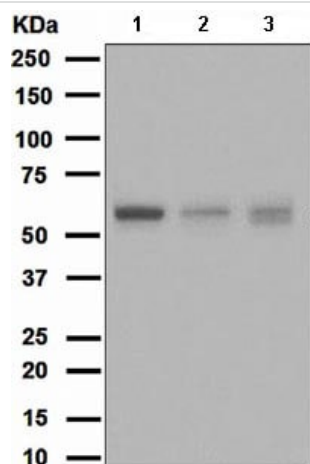
Lane 4: HCT116 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - [ab109491](#) observed at 55 kDa. Observed band shows ATG12-ATG5 conjugation. Red - loading control, [ab8245](#), 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. [Ab109491](#) and [ab8245](#) (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 IgG H&L (IRDye[®] 800CW) preabsorbed [ab216773](#) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed [ab216776](#) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ATG12 antibody [EPR4800]
(ab109491)

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

Lane 1 : Human fetal kidney tissue lysate

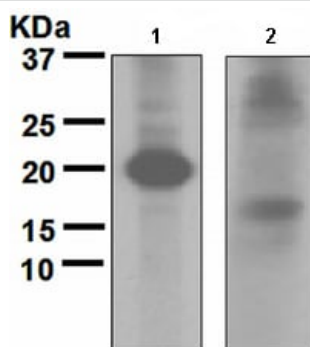
Lane 2 : HepG2 cell lysate

Lane 3 : Raji lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 15 kDa

Observed band shows ATG12-ATG5 conjugation



Western blot - Anti-ATG12 antibody [EPR4800]
(ab109491)

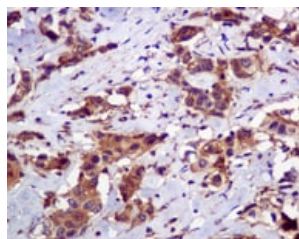
All lanes : Anti-ATG12 antibody [EPR4800] (ab109491) at 1/1000 dilution

Lane 1 : ATG12 transfected 293T lysate

Lane 2 : Non-transfected 293T lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 15 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG12 antibody [EPR4800] (ab109491)

ab109491, 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.

Why choose a recombinant antibody?

<p>Research with confidence Consistent and reproducible results</p>	<p>Long-term and scalable supply Recombinant technology</p>
<p>Success from the first experiment Confirmed specificity</p>	<p>Ethical standards compliant Animal-free production</p>

Anti-ATG12 antibody [EPR4800] (ab109491)

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

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