abcam

Product datasheet

Anti-LC3B antibody [EPR18709] - BSA and Azide free ab221794

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-LC3B antibody [EPR18709] - BSA and Azide free
Description	Rabbit monoclonal [EPR18709] to LC3B - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: BMDM, U-87 MG, C6 and RAW 264.7 whole cell lysates; Human brain, mouse heart, rat heart, mouse brain and rat brain lysates. ICC/IF: HeLa cells (+/- chloroquine), HAP1 cells (+/- chloroquine) (HAP1-MAP1LC3B knockout cells used as negative cell line); H9C2 rat cardiomyocytes. IHC-P: Human Cerebral Cortex tissue sections
General notes	ab221794 is the carrier-free version of **ab192890**. 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. 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® 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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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
Clonality	Monoclonal
Clone number	EPR18709
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab221794 in the following tested applications.

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

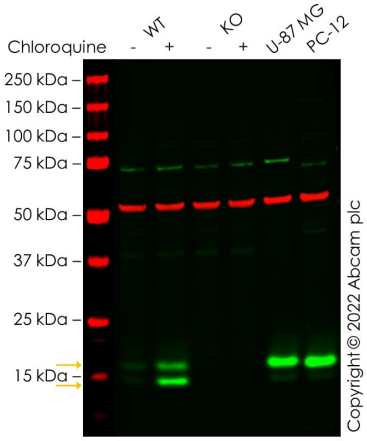
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 14, 16 kDa (predicted molecular weight: 15 kDa).
IHC-P		Use a concentration of 0.1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Function	Probably involved in formation of autophagosomal vacuoles (autophagosomes).
Tissue specificity	Most abundant in heart, brain, skeletal muscle and testis. Little expression observed in liver.
Sequence similarities	Belongs to the MAP1 LC3 family.
Post-translational modifications	The precursor molecule is cleaved by APG4B/ATG4B to form LC3-I. This is activated by APG7L/ATG7, transferred to ATG3 and conjugated to phospholipid to form LC3-II.
Cellular localization	Cytoplasm > cytoskeleton. Endomembrane system. Cytoplasmic vesicle > autophagosome membrane. LC3-II binds to the autophagic membranes.

Images



Western blot - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

All lanes : Anti-LC3B antibody [EPR18709] - Autophagosome Marker (**ab192890**) at 1/2000 dilution

Lane 1 : Wild-type HepG2 untreated control cell lysate
Lane 2 : Wild-type HepG2 Treated Chloroquine (50 uM, 16 h) cell lysate
Lane 3 : MAP1LC3B knockout HepG2 untreated control cell lysate
Lane 4 : MAP1LC3B knockout HepG2 Treated Chloroquine (50 uM, 16 h) cell lysate
Lane 5 : U-87 MG cell lysate
Lane 6 : PC-12 cell lysate

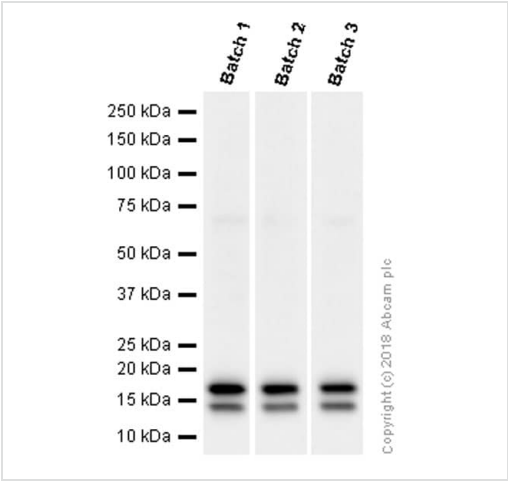
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 14,16 kDa

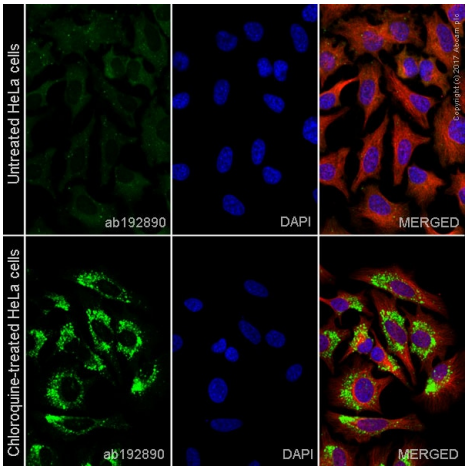
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192890**).

False colour image of Western blot: Anti-LC3B antibody [EPR18709] - Autophagosome Marker staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab192890** was shown to bind specifically to LC3B. A band was observed at 16/14 kDa (yellow arrows) in treated wild-type HepG2 cell lysates with no signal observed at this size in MAP1LC3B knockout cell line **ab277828** (knockout cell lysate **ab283796**). To generate this image, wild-type and MAP1LC3B knockout HepG2 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

This data was developed using **ab192890**, the same antibody clone in a different buffer formulation. Different batches of **ab192890** were tested onU-87 MG (Human glioblastoma-astrocytoma epithelial cell line) lysate at 0.9 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 14,16 kDa.

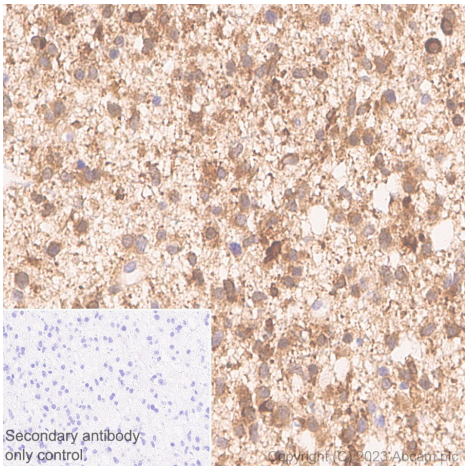


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

ab192890 staining LC3B in HeLa cells +/- Chloroquine (50µM, 24 hours). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab192890** at 1µg/ml and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red) followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

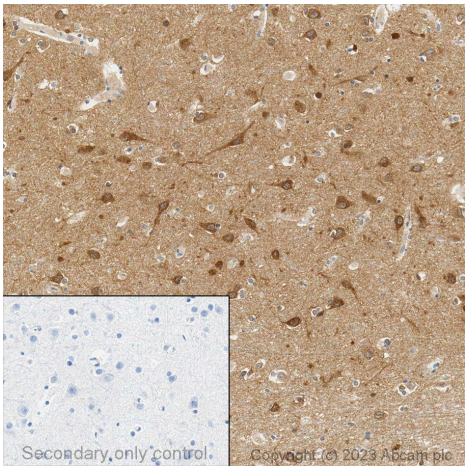
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192890**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192890**).

ab192890 staining LC3B in paraffin embedded human astrocytoma tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins. Samples were incubated with primary antibody at 1/1000 dilution for 30 mins at room temperature. Ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used as the secondary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192890**).

Immunohistochemical analysis of formalin fixed paraffin embedded human cortex labelling LC3B with **ab192890** at a dilution of 0.1 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5) . **ab192890** anti LC3B antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Tissue Microarray (TMA) data for ab192890

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	x	Clear cell carcinoma of human kidney	x	Human glioma	✓
Human cerebrum	✓	Human skeletal muscle	x	Human bladder cancer	x	Human hepatocellular carcinoma	x
Human colon	x	Human skin	x	Human breast carcinoma	x	Human lung carcinoma	x
Human endometrium	x	Human spleen	x	Human cervical carcinoma	x	Human ovarian carcinoma	x
Human kidney	x	Human stomach	x	Human colon carcinoma	x	Human pancreatic carcinoma	x
Human liver	x	Human testis	x	Human endometrial carcinoma	x	Human prostatic hyperplasia	x
Human lung	x	Human thyroid	x	Human gastric adenocarcinoma	x	Human thyroid carcinoma	x
Human mammary gland	x	Human tonsil	x				
Human pancreas	x						

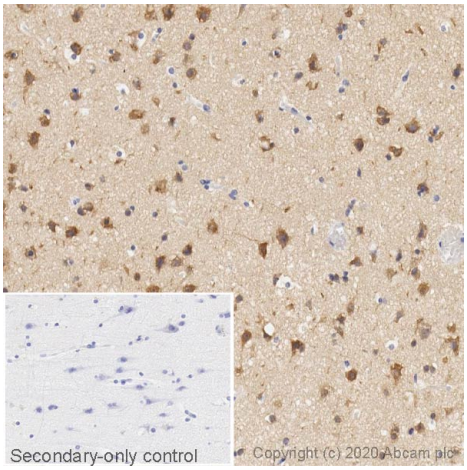
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192890**).

Tissue Microarrays stained for Anti-LC3B antibody [EPR18709] - Autophagosome Marker using **ab192890** in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested.

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.

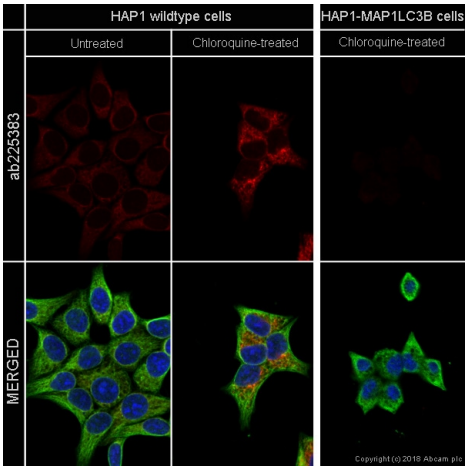
The section was incubated with **ab192890** for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

IHC image of LC3B staining in a section of formalin-fixed paraffin-embedded normal human cerebral cortex* performed on a Leica BOND™ system using the standard protocol **F**. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab221794, 0.1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

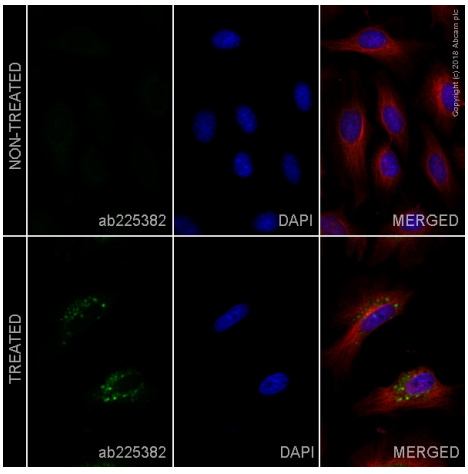


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

Clone EPR18709 (ab221794) has been successfully conjugated by Abcam. This image was generated using Anti-LC3B antibody [EPR18709] - Autophagosome Marker (Alexa Fluor® 647). Please refer to **ab225383** for protocol details.

ab225383 staining LC3B in wild-type HAP1 cells and knockout cells, untreated and chloroquine-treated (50µM, 24 hours). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab225383** at 0.5µg/ml (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

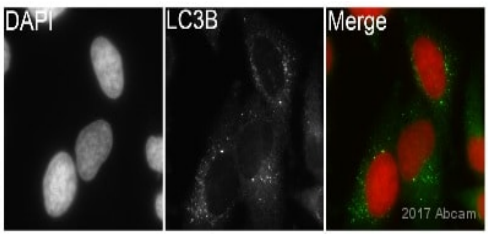


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

Clone EPR18709 (ab221794) has been successfully conjugated by Abcam. This image was generated using Anti-LC3B antibody [EPR18709] - Autophagosome Marker (Alexa Fluor® 488). Please refer to **ab225382** for protocol details.

ab225382 staining LC3B in HeLa chloroquine-treated (50µM, 24 hours) cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab225453** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

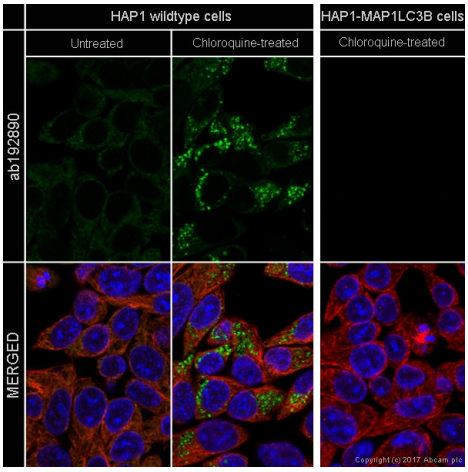


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

This image is courtesy of an Abreview submitted by Kirk McManus, Univ. of Manitoba/Cancer Care MICB.

Immunocytochemistry/ Immunofluorescence analysis of HeLa cells labeling LC3B with **ab192890** at 1/500 dilution. Cells were fixed in Methanol. Staining with **ab192890** at 1/500 was carried out for 1 hour at 22°C in PBS buffer. **ab150081**, a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody was used at 1/200 dilution. DAPI was used to counterstain.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192890**).

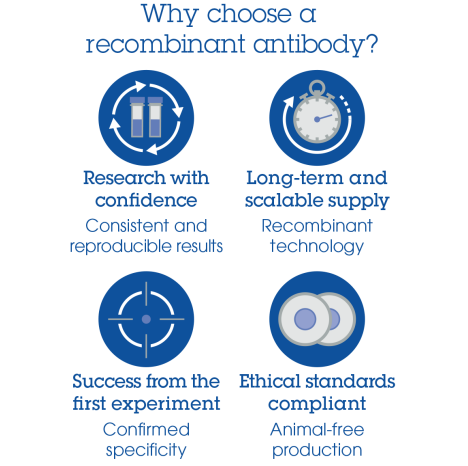


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

This ICC data was generated using the same anti-LC3B antibody clone, EPR18709, in a different buffer formulation (cat# **ab192890**).

ab192890 staining LC3B in HAP1 cells (wildtype and MAP1LC3B knockout) +/- Chloroquine (50µM, 24 hours). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab192890** at 1µg/ml and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red) followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Anti-LC3B antibody [EPR18709] - BSA and Azide free (ab221794)

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