abcam

Product datasheet

Anti-LC3B antibody [EPR18709] - Autophagosome Marker ab192890

KO VALIDATED Recombinant RabMAb

17 Abreviews 305 References 12 Images

Overview

Product name	Anti-LC3B antibody [EPR18709] - Autophagosome Marker
Description	Rabbit monoclonal [EPR18709] to LC3B - Autophagosome Marker
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, 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). IHC-P: Human Cerebral Cortex tissue sections
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18709
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab192890 in the following tested applications.

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

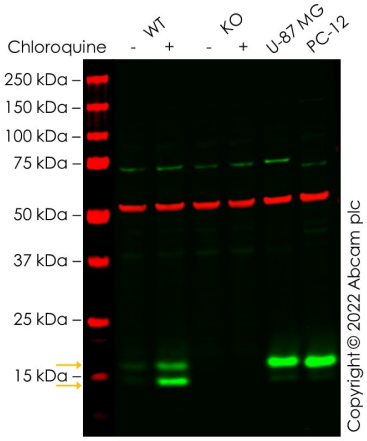
Application	Abreviews	Notes
IHC-P		Use a concentration of 0.1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★★ **(8)**	1/2000. Detects a band of approximately 14, 16 kDa (predicted molecular weight: 15 kDa).
ICC/IF	★★★★★ **(6)**	Use a concentration of 1 µg/ml.

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] - Autophagosome Marker (ab192890)

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

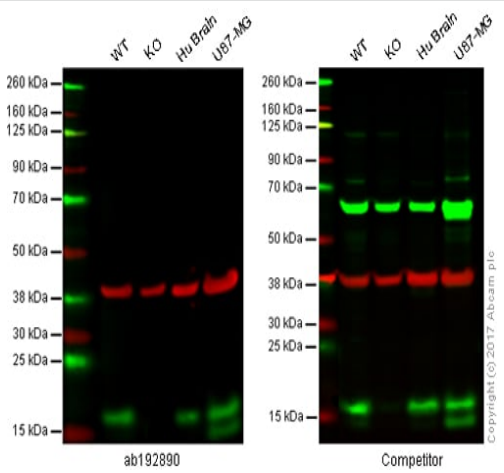
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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.

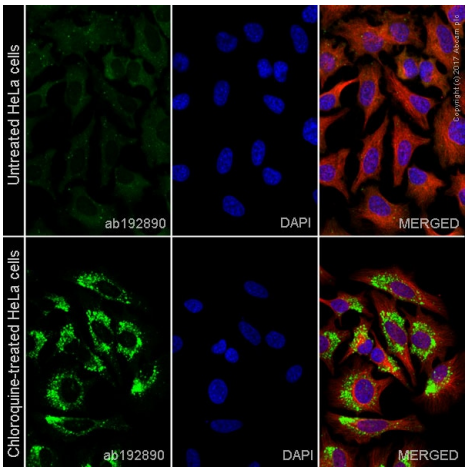


Western blot - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: LC3B knockout HAP1 cell lysate (20 µg)
Lane 3: Human brain tissue lysate (20 µg)
Lane 4: U-87 MG cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green).

Green -target observed at 14 and 16 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab192890 and a competitor's top cited rabbit polyclonal antibody.



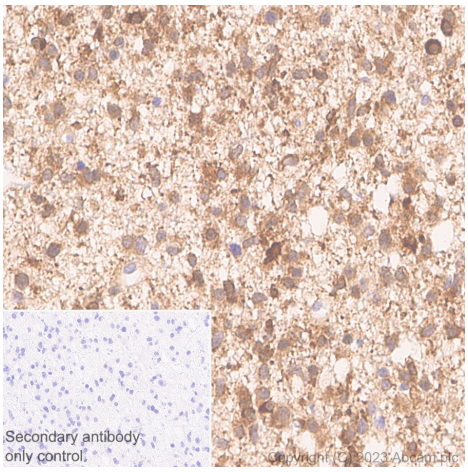
Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

ab192890 staining LC3B in HeLa (Human epithelial cell line from cervix adenocarcinoma) 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 labeled with DAPI (shown in blue).

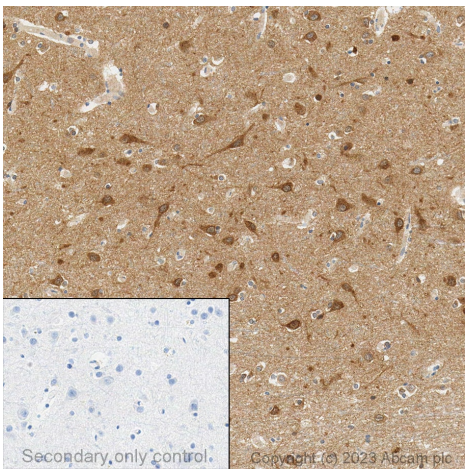
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Alexa Fluor® 488 (**ab225383**) and Alexa Fluor® 647 (**ab225382**) conjugated versions are available for this clone.



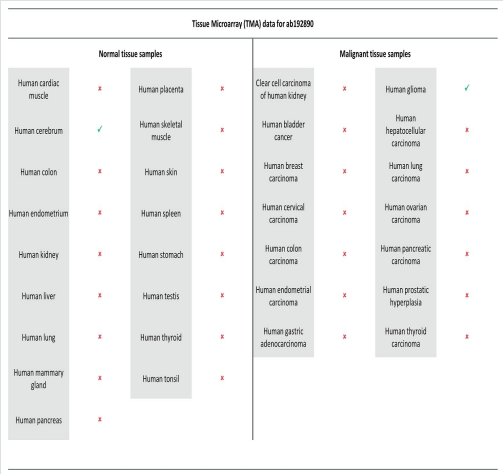
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

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] - Autophagosome Marker (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

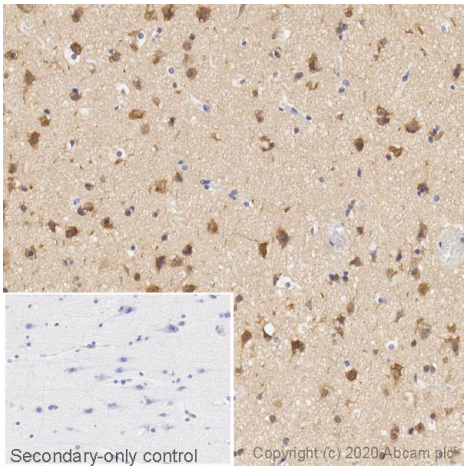
Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	x	Clear cell carcinoma of human kidney	x	Human glioma	✓
Human cerebrum	✓	Human skeletal muscle	x	Human bladder cancer	x	Human hepatocellular carcinoma	x
Human colon	x	Human skin	x	Human breast carcinoma	x	Human 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] - Autophagosome Marker (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.

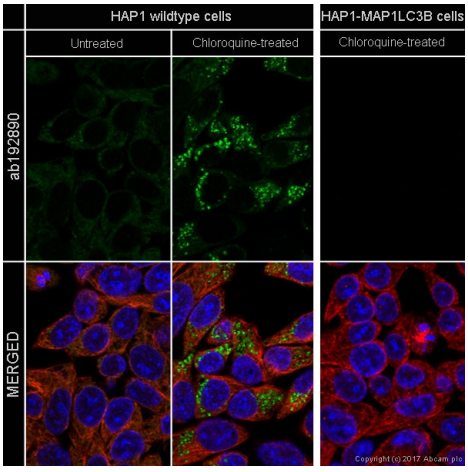


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

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.

This data was developed using the same antibody clone in a different buffer formulation containing only PBS (**ab221794**).

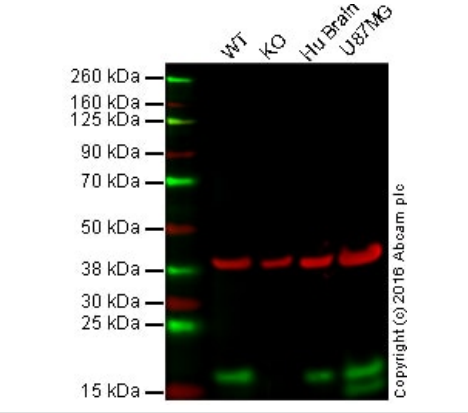


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

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 1 µg/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 labeled with DAPI (shown in blue).

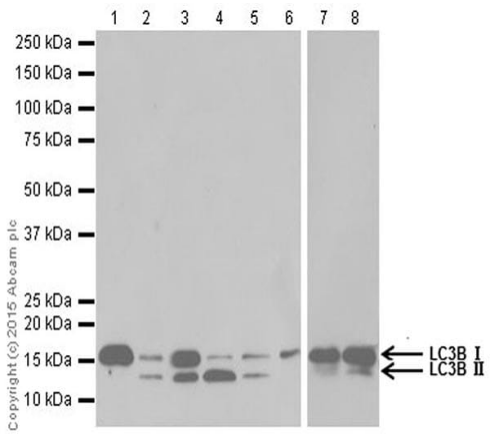
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: LC3B knockout HAP1 cell lysate (20 µg)
Lane 3: Human brain tissue lysate (20 µg)
Lane 4: U-87 MG cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab192890 observed at 14 and 16 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab192890 was shown to specifically react with LC3B in wild-type HAP1 cells. No band was observed when LC3B knockout samples were examined. Wild-type and LC3B knockout samples were subjected to SDS-PAGE. ab192890 and **ab8245** (loading control to GAPDH) were diluted 1/2000 and 1/10,000 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 hour at room temperature before imaging.



Western blot - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

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

Lane 1 : Human brain lysate
Lane 2 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate
Lane 3 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 4 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 5 : Mouse heart lysate
Lane 6 : Rat heart lysate
Lane 7 : Mouse brain lysate
Lane 8 : Rat brain lysate

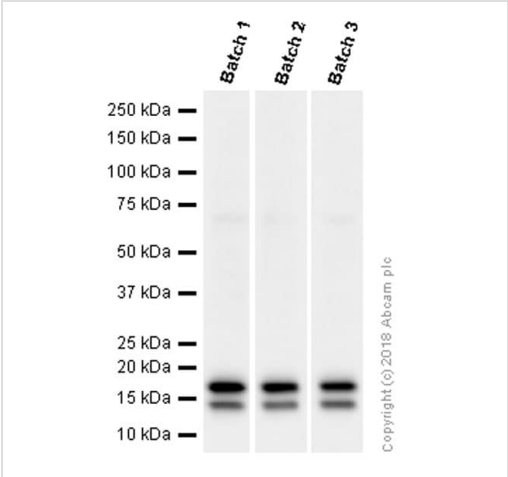
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1-6: 3 minutes; Lane 7 and 8: 30 seconds.



Western blot - Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

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.

Anti-LC3B antibody [EPR18709] - Autophagosome Marker (ab192890)

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