**Overview**

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-LC3B antibody - Autophagosome Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit polyclonal to LC3B - Autophagosome Marker</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: ICC/IF, IHC-P, WB</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Human</td>
</tr>
<tr>
<td><strong>Predicted to work with</strong></td>
<td>Cow</td>
</tr>
</tbody>
</table>

**Immunogen**

Synthetic peptide corresponding to Human LC3B (N terminal). A synthetic peptide made to an N-terminal portion of the human LC3 protein sequence (between residues 1-100). Database link: [Q9GZQ8](Q9GZQ8)

**Positive control**

- WB: HeLa, NIH/3T3 cells.
- ICC/IF: HeLa cells; rat hepatocyte cells.
- IHC-P: Mouse brain tissue.

**General notes**

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

**Properties**

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
</tr>
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<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.</td>
</tr>
<tr>
<td><strong>Storage buffer</strong></td>
<td>pH: 7.40</td>
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<tr>
<td></td>
<td>Preservative: 0.02% Sodium azide</td>
</tr>
<tr>
<td></td>
<td>Constituent: PBS</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
</tbody>
</table>

**Applications**

- ICC/IF
- IHC-P
- WB
The Abpromise guarantee

Our Abpromise guarantee covers the use of ab48394 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>🟢🟢🟢🟢🟢 (4)</td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>🟢🟢🟢🟢🟢 (3)</td>
<td>1/200 - 1/400. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Antigen Retrieval: Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.</td>
</tr>
<tr>
<td>WB</td>
<td>🟢🟢🟢🟢🟢 (20)</td>
<td>Use a concentration of 0.5 - 2 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa). Detects bands of approximately 17 kDa (LC3-II) and 19 kDa (LC3-I).</td>
</tr>
</tbody>
</table>

**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**

All lanes: Anti-LC3B antibody - Autophagosome Marker (ab48394) at 0.5 µg/ml

Lane 1: Wild-type HepG2 untreated control cell lysate
Lane 2: Wild-type HepG2 Treated Chloroquine (50 µM, 16 h) cell lysate
Lane 3: MAP1LC3B knockout HepG2 untreated control cell lysate
Lane 4: MAP1LC3B knockout HepG2 Treated Chloroquine (50 µM, 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 - Autophagosome Marker staining at 0.5 μg/ml, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab48394 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.

Immunostaining of LC3 (green) in the arcuate hypothalamic nucleus of lean and obese mice (A; 8 weeks of an HFD or B; 16 weeks of an HFD). DAPI (blue) was used for nuclear staining.

The brain was excised after the mice were decapitated. Each SNC was fixed in 4% paraformaldehyde and each hypothalamus was processed for paraffin embedding and sectioned into 5.0 μm sections. Samples were incubated with primary antibodies overnight and with secondary antibodies conjugated to FITC or rhodamine for 2 hours (sc2777 and sc2092, respectively; Santa Cruz Biotechnology, Santa Cruz, CA). The DAPI stain was used for nuclear staining while the Leica FW 4500 B microscope captured the images. Hypothalamic areas were observed according to the landmarks in the mouse brain atlas. Analysis and documentation of the results were performed using Leica Application Suite V3.6 (Switzerland).
Western Blot shows lysates of HeLa (human epithelial cell line from cervix adenocarcinoma) cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50 µM Chloroquine for 18 hours. PVDF membrane was probed with 0.5 µg/mL ab48394 followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody. A specific band was detected for LC3B at approximately 15 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. GAPDH is shown as a loading control. This experiment was conducted under reducing conditions.

HeLa (human epithelial cell line from cervix adenocarcinoma) cells (wild type, left; LC3B knockout HeLa, right) stained for LC3B using ab48394 (red) at 0.3 µg/ml in ICC/IF. Primary antibody was incubated for 3 hours at room temperature, followed by NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody. Counterstained with DAPI (blue).

LC3 was detected in immersion fixed Chloroquine treated Hela cells (left) but was not detected in LC3 knockout Hela cells (right).
LC3B immunofluorescence in primary follicles of PD 13 ovaries. The red dots represent LC3b and DAPI (blue) indicated cell nuclei.

For the immunofluorescence analysis, the ovaries were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned into 5 μm slices. After antigen retrieval, the slides were blocked with goat serum and incubated with primary antibody (rabbit anti-LC3B at 1:200) overnight at 4°C. Alexa Fluor 594 (Invitrogen) was used as the secondary antibody in immunofluorescence assays.

Formalin-fixed, paraffin-embedded mouse brain tissue stained for LC3B using ab48394 at 1/200 dilution in immunohistochemical analysis. The specific signal of LC3 was detected using HRP-conjugated secondary antibody with DAB reagent, and nuclei of cells were counterstained using hematoxylin. This LC3 antibody generated a low to moderate levels of cytoplasmic staining in the glial cells. The neurons depicted a moderate to strong staining for LC3 in their cytoplasm.
Western blot shows lysates of mouse NIH/3T3 (mouse embryo fibroblast cell line) and rat PC-12 (rat adrenal gland pheochromocytoma cell line) cell lines untreated (-) or treated (+) with Chloroquine. PVDF membrane was probed with 0.5 ug/mL rabbit anti-LC3B polyclonal Antibody (ab48394), followed by 1:2000 dilution of goat anti-rabbit IgG secondary antibody.

ab48394 staining LC3B in a Rat hepatocyte by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde, permeabilized with 0.2% Triton X-100 in PBS and blocked with 1% Donkey serum in 0.1% PBST for 60 minutes at 21°C. Samples were incubated with primary antibody (1/50 in PBS + 1% BSA) for 3 hours at 22°C. An Alexa Fluor® 394-conjugated Donkey anti-rabbit IgG polyclonal was used as the secondary antibody at a dilution of 1/200.
**Western blot** - Anti-LC3B antibody - Autophagosome Marker (ab48394)

This image is courtesy of an anonymous Abreview

**All lanes**: Anti-LC3B antibody - Autophagosome Marker (ab48394) at 1/2000 dilution

**Lane 1**: Rat whole tissue lysate - Normal liver

**Lane 2**: Rat whole tissue lysate - liver treated with AEE788 at 50 mg/kg 3 times a week for 1 week

**Lane 3**: Rat whole tissue lysate - liver treated with RAD at 2.5 mg/kg daily for 1 week

Lysates/proteins at 30 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Developed using the ECL technique.

Performed under non-reducing conditions.

**Predicted band size**: 15 kDa

**Observed band size**: 17 kDa

**Exposure time**: 1 minute

ab48394 staining LC3B in HeLa cells treated with calmidazolium chloride (ab120658), by ICC/IF. Increase of LC3B expression correlates with increased concentration of calmidazolium chloride, as described in literature.

The cells were incubated at 37°C for 6h in media containing different concentrations of ab120658 (calmidazolium chloride) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab48394 (1 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

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