abcam

Product datasheet

Anti-LC3B antibody - Autophagosome Marker ab48394

31 Abreviews 625 References 画像数 10

製品の概要

製品名	Anti-LC3B antibody - Autophagosome Marker
製品の詳細	Rabbit polyclonal to LC3B - Autophagosome Marker
由来種	Rabbit
アプリケーション	**適用あり:** ICC/IF, IHC-P, WB
種交差性	**交差種:** Mouse, Rat, Human **交差が予測される動物種:** Cow
免疫原	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
ポジティブ・コントロール	WB: HeLa, NIH/3T3 cells. ICC/IF: HeLa cells; rat hepatocyte cells. IHC-P: Mouse brain tissue.
特記事項	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

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
精製度	Immunogen affinity purified
ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab48394の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF	★★★★★ (4)	Use a concentration of 1 µg/ml.
IHC-P	★★★★☆ (3)	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.
WB	★★★★☆ (20)	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).

ターゲット情報

機能 Probably involved in formation of autophagosomal vacuoles (autophagosomes).

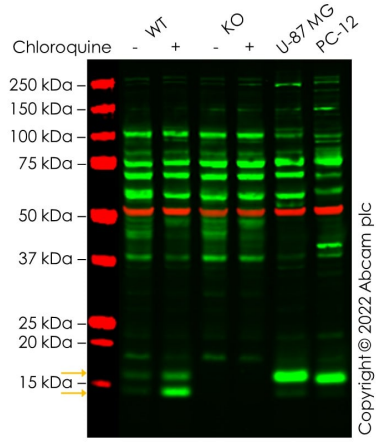
組織特異性 Most abundant in heart, brain, skeletal muscle and testis. Little expression observed in liver.

配列類似性 Belongs to the MAP1 LC3 family.

翻訳後修飾 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.

細胞内局在 Cytoplasm > cytoskeleton. Endomembrane system. Cytoplasmic vesicle > autophagosome membrane. LC3-II binds to the autophagic membranes.

画像



Western blot - Anti-LC3B antibody - Autophagosome Marker (ab48394)

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 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

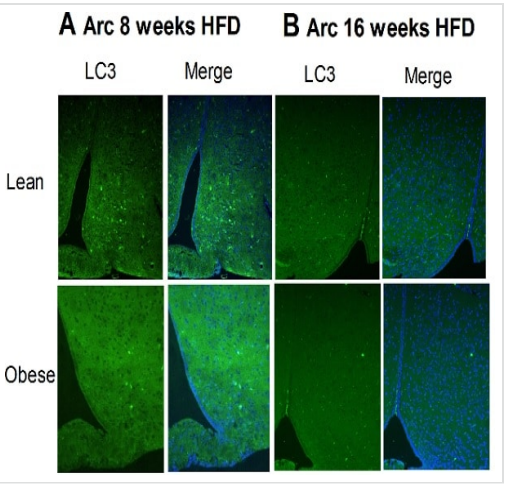
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 14,16 kDa

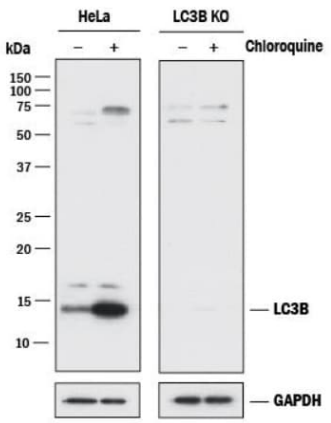
False colour image of Western blot: Anti-LC3B antibody - Autophagosome Marker staining at 0.5 ug/ml, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab48394 was shown to bind specifically to LC3B. A band was observed 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody - Autophagosome Marker (ab48394)

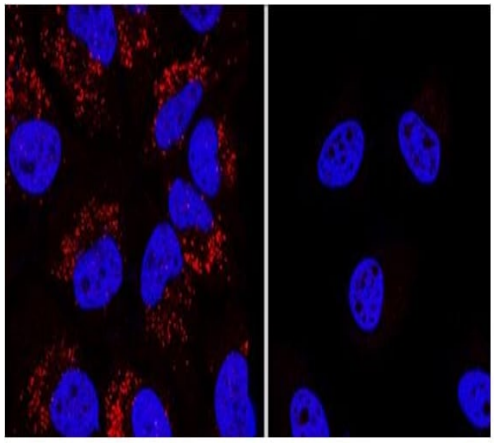
Immunostaining of LC3 (green) in the arcuate hypothalamic nucleus of lean and obese mice (A; 8 weeks of a HFD or B; 16 weeks of a HFD). DAPI (blue) was used for nuclear staining.

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 (sc2777and 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 - Anti-LC3B antibody - Autophagosome Marker (ab48394)

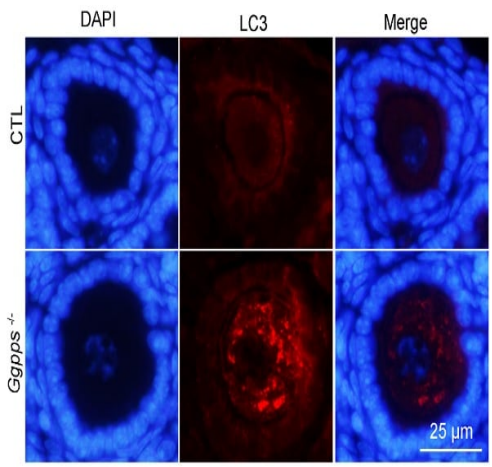
Western Blot shows lysates of HeLa (human epithelial cell line from cervix adenocarcinoma) cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50 uM Chloroquinine for 18 hours. PVDF membrane was probed with 0.5 ug/mL ab48394 followed by HRP-conjugated Anti-Rabbit IgG Secondary 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.



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody - Autophagosome Marker (ab48394)

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).

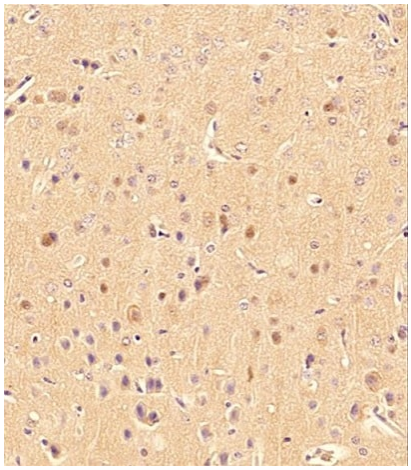
LC3 was detected in immersion fixed Cloroquine treated Hela cells (left) but was not detected in LC3 knockout Hela cells (right).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody - Autophagosome Marker (ab48394)

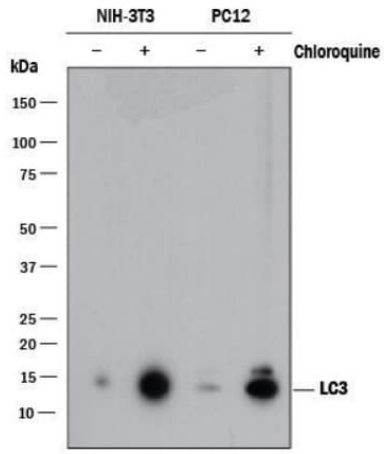
LC3B immunofluorescence in primary follicles of PD 13 ovaries. The red dots represent LC3b and DAPI (blue) indicated cell nuclei.

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.



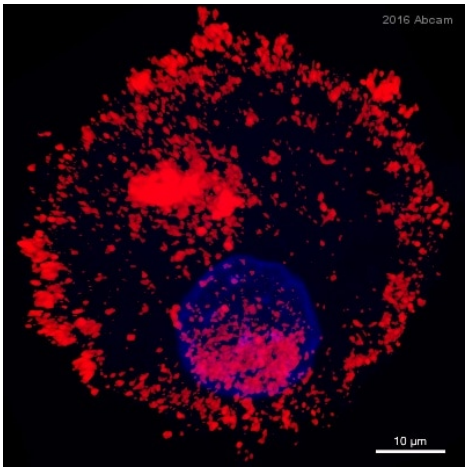
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody - Autophagosome Marker (ab48394)

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 - Anti-LC3B antibody - Autophagosome Marker (ab48394)

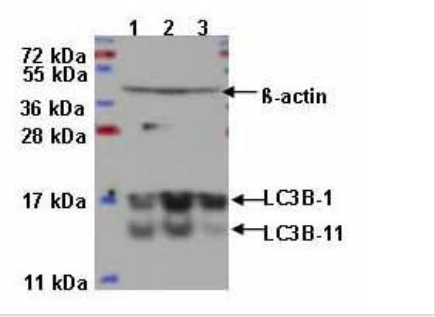
Western blot shows lysates of mouse NIH/3T3 (mouse embryo fibroblast cell line) and rat PC-12 (rat adrenal gland pheochromocytoma cell line) cell lines untreated (-) or treated (+) with Chloroquine. PVDF membrane was probed with 0.5 ug/mL rabbit anti-LC3B polyclonal Antibody (ab48394), followed by 1:2000 dilution of goat anti-rabbit IgG secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody - Autophagosome Marker (ab48394)

This image is courtesy of an Abreview by Armen Petrosyan.

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.

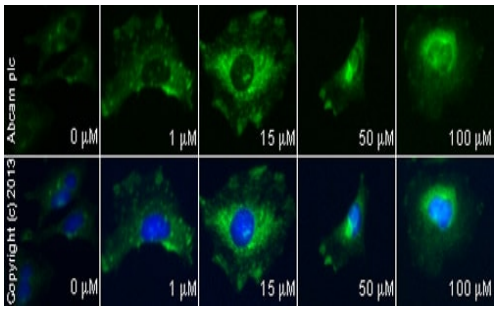
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/5000 dilution

Developed using the ECL technique.

Performed under non-reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

Exposure time: 1 minute



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody - Autophagosome Marker (ab48394)

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.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.co.jp/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors