abcam

Product datasheet

Anti-LC3B antibody ab63817

敲除验证

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概述

产品名称	Anti-LC3B抗体
描述	**兔多克隆抗体**to LC3B
宿主	Rabbit
经测试应用	**适用于:** IHC-P, WB, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human **预测可用于:** Chicken, Cow, Macaque monkey, Zebra finch
免疫原	Synthetic peptide corresponding to Human LC3B aa 1-100 (N terminal) conjugated to keyhole limpet haemocyanin. Peptide available as **ab87823**. Database link: **Q9GZQ8** (Peptide available as **ab87823**)
阳性对照	This antibody gave a positive signal in Human, Mouse and Rat Brain Tissue Lysate. ICC/IF: HeLa cells (+/- chloroquine), HAP1 cells (+/-chloroquine) (HAP1-MAP1LC3B knockout cells used as negative cell line).
常规说明	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

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab63817于以下的经测试应用**

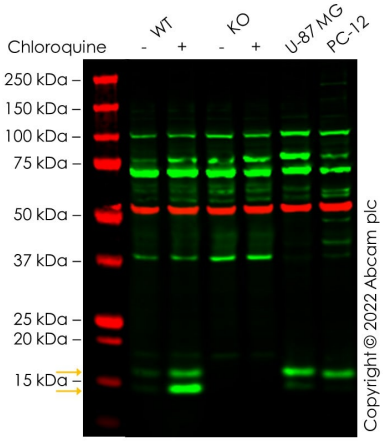
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ICC/IF	★★★☆☆ (1)	Use a concentration of 1 µg/ml.

靶标

功能	Probably involved in formation of autophagosomal vacuoles (autophagosomes).
组织特异性	Most abundant in heart, brain, skeletal muscle and testis. Little expression observed in liver.
序列相似性	Belongs to the MAP1 LC3 family.
翻译后修饰	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.
细胞定位	Cytoplasm > cytoskeleton. Endomembrane system. Cytoplasmic vesicle > autophagosome membrane. LC3-II binds to the autophagic membranes.

图片



Western blot - Anti-LC3B antibody (ab63817)

All lanes : Anti-LC3B antibody (ab63817) at 1 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

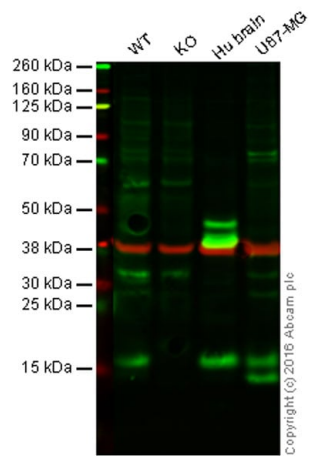
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

False colour image of Western blot: Anti-LC3B antibody staining at 1 ug/ml, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab63817 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,

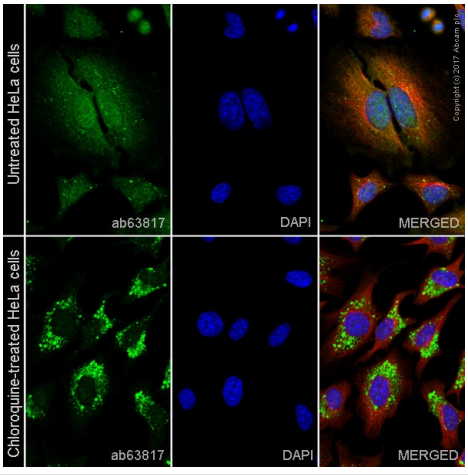
washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-LC3B antibody (ab63817)

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: U87MG cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab63817 observed at 16 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

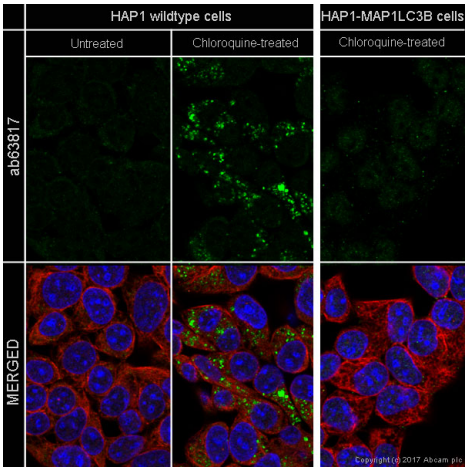
ab63817 was shown to recognize LC3B in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when LC3B knockout samples were examined. Wild-type and LC3B knockout samples were subjected to SDS-PAGE. ab63817 and **ab8245** (loading control to GAPDH) were diluted 1 µg/mL 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.



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody (ab63817)

ab63817 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 ab63817 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).



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody (ab63817)

ab63817 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 ab63817 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).

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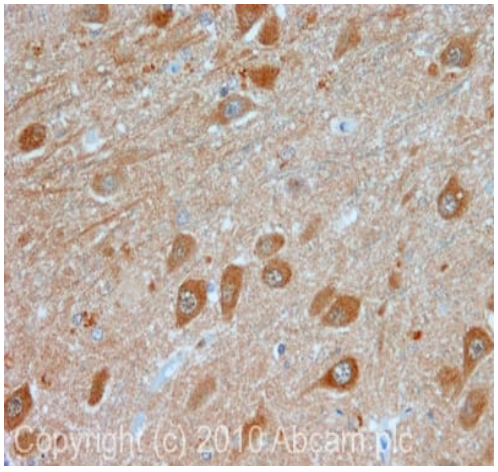
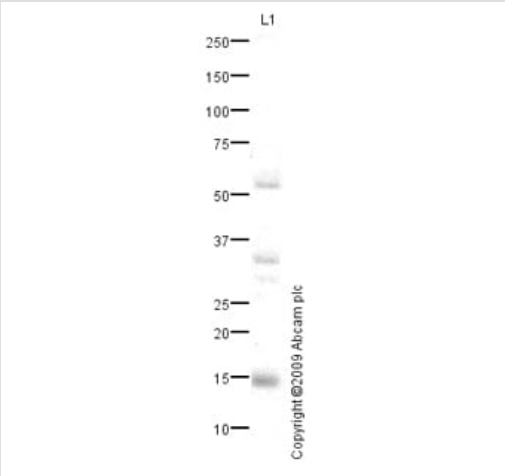


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LC3B antibody (ab63817)

IHC image of LC3B staining in human hippocampus formalin fixed paraffin embedded tissue section, 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 20 mins. The section was then incubated with ab63817, 1µg/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.



Western blot - Anti-LC3B antibody (ab63817)

Anti-LC3B antibody (ab63817) at 1 µg/ml + Human brain tissue lysate - total protein (**ab29466**) at 10 µg

Secondary

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 15 kDa
Additional bands at: 32 kDa, 54 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes

Western blot - Anti-LC3B antibody (ab63817)

All lanes : Anti-LC3B antibody (ab63817) at 1 µg/ml

Lane 1 : Brain (Mouse) Tissue Lysate
Lane 2 : Brain (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

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

Additional bands at: 30 kDa, 65 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 3 minutes

Western blot - Anti-LC3B antibody (ab63817)

Anti-LC3B antibody (ab63817) at 1 µg/ml + LC3B protein (Human) (**ab87944**) at 0.1 µg

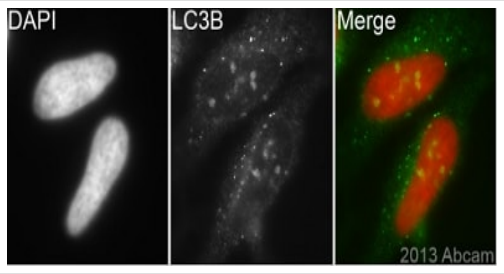
Secondary
Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 2 minutes



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody (ab63817)

This image is courtesy of an Abreview submitted by Kirk Mcmanus.

Immunocytochemical analysis of HeLa cells, labeling LC3B with ab63817 (1/200 with PBS for 16 hours at 22°C). Cells were methanol fixed, immunofluorescently labeled, adn counterstained with DAPI.

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