abcam

Product datasheet

Anti-LC3B antibody - N-terminal ab229327

10 References 14 Images

Overview

Product name Anti-LC3B antibody - N-terminal

Description Rabbit polyclonal to LC3B - N-terminal

Host species Rabbit

Tested applications Suitable for: WB, Flow Cyt, ICC/IF, IP, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow, Chimpanzee, Zebrafish, Rhesus monkey, Medaka fish

Immunogen Synthetic peptide within Human LC3B (N terminal). The exact sequence is proprietary. (Carrier-

protein conjugated).

Database link: **Q9GZQ8**

Positive control ICC/IF: HeLa cells untreated and treated with 50µM Chloroquine for 24 hr; Hep G2 cells untreated

and treated with 3 μ M Thapsigargin for 12 hrs. WB: Huh7 whole cell lysate; NTERA-2 cl.D1 [NT2/D1], PC-3, U-87 MG and SK-N-SH whole cell extracts; Mouse and rat brain lysates; Hep G2 cell lysate untreated and treated with 3 μ M Thapsigargin; HeLa whole cell extract untreated and treated with 50 μ M Chloroquine for 24 hr. IHC-P: Mouse brain tissue. IP: U-87 MG whole cell

extract. Flow Cyt: HeLa cells.

General notesThe 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

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: 7.00

Preservative: 0.025% Proclin 300

Constituents: 78% PBS, 1% BSA, 20% Glycerol (glycerin, glycerine)

1

Purity Immunogen affinity purified

Polyclonal Clonality

Isotype ΙgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab229327 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		1/500 - 1/3000. Predicted molecular weight: 15 kDa.
Flow Cyt		1/50 - 1/200.
ICC/IF		1/100 - 1/1000.
IP		1/100 - 1/500.
IHC-P		1/100 - 1/1000.

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

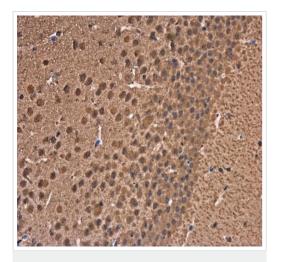
The precursor molecule is cleaved by APG4B/ATG4B to form LC3-I. This is activated by

APG7L/ATG7, transferred to ATG3 and conjugated to phospholipid to form LC3-II.

Cellular localization Cytoplasm > cytoskeleton. Endomembrane system. Cytoplasmic vesicle > autophagosome

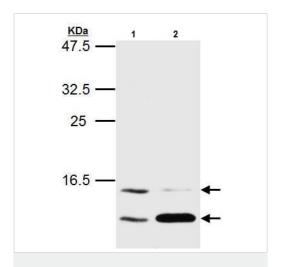
membrane. LC3-II binds to the autophagic membranes.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LC3B antibody - N-terminal (ab229327)

Paraffin-embedded mouse brain tissue stained for LC3B using ab229327 at 1/500 dilution in immunohistochemical analysis.



Western blot - Anti-LC3B antibody - N-terminal (ab229327)

All lanes : Anti-LC3B antibody - N-terminal (ab229327) at 1/1500 dilution

Lane 1: Huh7 whole cell lysate (untreated)

Lane 2 : Huh7 whole cell lysate (3 µM Thapsigargin treatment for

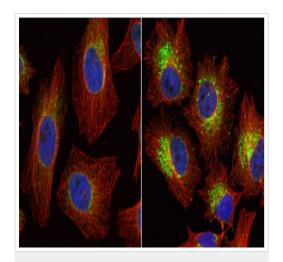
12 hr)

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

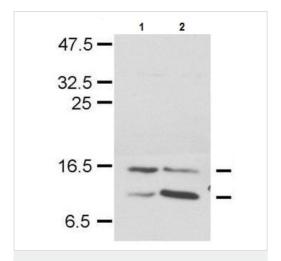


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody - N-terminal (ab229327)

4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells, mock treated (left panel) or treated with 50 μ M Chloroquine for 24 hr (right panel), stained for LC3B (green) using ab229327 at 1/2000 dilution in ICC/IF.

Red: alpha Tubulin, a cytoskeleton marker, stained by alpha Tubulin antibody at 1/1000 dilution.

Blue: Hoechst 33342 staining.



Western blot - Anti-LC3B antibody - N-terminal (ab229327)

All lanes : Anti-LC3B antibody - N-terminal (ab229327) at 1/1500 dilution

Lane 1 : Huh7 whole cell lysate (un-infected)

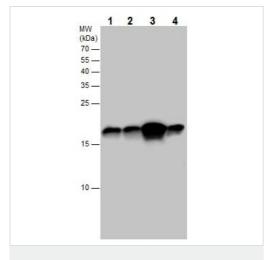
Lane 2 : Huh7 whole cell lysate (HCV-infected)

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa



Western blot - Anti-LC3B antibody - N-terminal (ab229327)

All lanes : Anti-LC3B antibody - N-terminal (ab229327) at 1/1000 dilution

Lane 1 : NTERA-2 cl.D1 [NT2/D1] (human malignant pluripotent embryonic carcinoma cell line) whole cell extract

Lane 2 : PC-3 (human prostate adenocarcinoma cell line) whole cell extract

Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell extract

Lane 4: SK-N-SH (human neuroblastoma cell line) whole cell extract

Lysates/proteins at 30 µg per lane.

Secondary

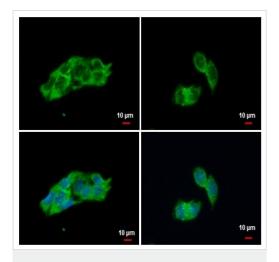
All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

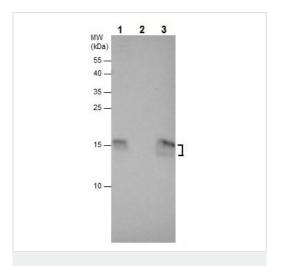
15% SDS-PAGE gel.

lce-cold methanol-fixed, ice-cold acetone permeabilized HepG2 (human liver hepatocellular carcinoma cell line) cells, mock treated (left panels) or treated with with 3 μM Thapsigargin for 12 hrs (right panels), stained for LC3B (green) using ab229327 at 1/500 dilution in ICC/IF.

Blue: Hoechst 33342 staining.



Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody - N-terminal (ab229327)



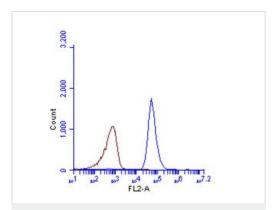
Immunoprecipitation - Anti-LC3B antibody - Nterminal (ab229327)

LC3B was immunoprecipitated from U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell extract (1 mg for IP, 20% of IP loaded) with 5 µg ab229327. Western blot was performed from the immunoprecipitate using ab229327. Anti-Rabbit IgG was used as a secondary reagent.

Lane 1: U-87 MG whole cell extract.

Lane 2: Control IgG IP in U-87 MG whole cell extract.

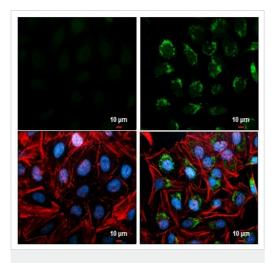
Lane 3: ab229327 IP in U-87 MG whole cell extract.



Flow Cytometry - Anti-LC3B antibody - N-terminal (ab229327)

Flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling LC3B with ab229327 at 1/100 dilution (blue) compared with an unlabelled sample (brown).

Acquisition of >20,000 events were collected using Argon ion laser (488nm) and 525/30 bandpass filter.

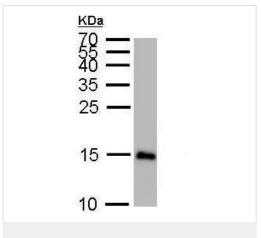


Immunocytochemistry/ Immunofluorescence - Anti-LC3B antibody - N-terminal (ab229327)

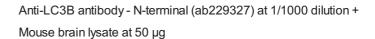
4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells, mock treated (left panels) or treated with 50 μ M Chloroquine for 24 hr (right panels), stained for LC3B (green) using ab229327 at 1/200 dilution in ICC/IF.

Red: phalloidin, a cytoskeleton marker, at 1/50 dilution.

Blue: Hoechst 33342 staining.



Western blot - Anti-LC3B antibody - N-terminal (ab229327)

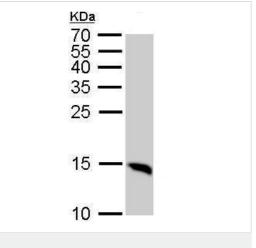


Secondary

HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

15% SDS-PAGE gel.



Western blot - Anti-LC3B antibody - N-terminal (ab229327)

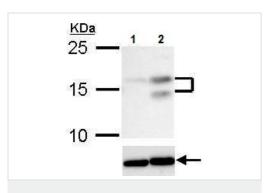
Anti-LC3B antibody - N-terminal (ab229327) at 1/1000 dilution + Rat brain lysate at 50 μg

Secondary

HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

15% SDS-PAGE gel.



Western blot - Anti-LC3B antibody - N-terminal (ab229327)

All lanes : Anti-LC3B antibody - N-terminal (ab229327) at 1/1000 dilution

Lane 1 : Untreated HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 2 : HepG2 whole cell lysate treated with 3 μ M thapsigargin for 12 hours

Lysates/proteins at 30 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

Lower panel: Beta-actin antibody at 1/20000 dilution.

15% SDS-PAGE gel.

1 2 3
MW (kDa)
35 —
25 —
15 —

Western blot - Anti-LC3B antibody - N-terminal

(ab229327)

All lanes : Anti-LC3B antibody - N-terminal (ab229327) at 1/1000 dilution

Lane 1 : Untreated HepG2 (human liver hepatocellular carcinoma cell line) whole cell extract

Lane 2 : HepG2 whole cell extract treated with 3 μ M thapsigargin for 16 hours

Lane 3 : HepG2 whole cell extract treated with 3 μ M thapsigargin for 24 hours

Lysates/proteins at 30 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

15% SDS-PAGE gel.

1 2
MW (kDa)
72 —
55 —
43 —
34 —
26 —

17 —

Western blot - Anti-LC3B antibody - N-terminal (ab229327)

All lanes : Anti-LC3B antibody - N-terminal (ab229327) at 1/2500 dilution

Lane 1 : Untreated HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell extract

Lane 2 : HeLa whole cell extract treated with $50\mu M$ Chloroquine for 24 hours

Lysates/proteins at 30 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 15 kDa

15% SDS-PAGE gel.

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