Anti-LC3B Antibody [JJ090-6]

ET1701-65

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IF-Tissue, IP, mIHC
Molecular Wt:	Predicted band size: 14/16 kDa
Clone number:	JJ090-6

Description: Microtubule-associated proteins (MAPs) regulate microtubule stability and play critical roles in neuronal development and in maintaining the balance between neuronal plasticity and rigidity. MAP-light chain 3 beta (MAP-LC3β) and MAP-light chain 3 alpha (MAP-LC3α) are subunits of both MAP1A and MAP1B. MAP-LC3β, a homolog of Apg8p, is essential for autophagy and associated to the autophagosome membranes after processing. Two forms of LC3β, the cytosolic LC3-I and the membrane-bound LC3-II, are produced post-translationally. LC3-I is formed by the removal of the C-terminal 22 amino acids from newly synthesized LC3β, followed by the conversion of a fraction of LC3-I into LC3-II. LC3 enhances fibronectin mRNA translation in ductus arteriosus cells through association with 60S ribosomes and binding to an AU-rich element in the 3' untranslated region of fibronectin mRNA. This facilitates sorting of fibronectin mRNA onto rough endoplasmic reticulum and translation. MAP LC3β may also be involved in formation of autophagosomal vacuoles. It is expressed primarily in heart, testis, brain and skeletal muscle.

Immunogen:	Synthetic peptide within human LC3 B aa 1-20.
Positive control:	HeLa cells treated with 50μM Chloroquine for 24 hours, HeLa cell lysate, HeLa treated with 50μM Chloroquine for 18 hours cell lysate, C2C12 cell lysate, C2C12 treated with 50μM Chloroquine for 18 hours cell lysate, C6 cell lysate, C6 treated with 50μM Chloroquine for 18 hours cell lysate, mouse brain tissue lysate, rat brain tissue lysate, HCT 116 cell lysate, HCT 116 treated with 50μM Chloroquine for 18 hours cell lysate, U-87 MG cell lysate, C2C12 cells treated with 50μM Chloroquine for 24 hours, C6 cells treated with 50μM Chloroquine for 24 hours, mouse brain tissue, mouse hippocampus tissue, rat brain tissue, rat hippocampus tissue.
Subcellular location:	Cytoplasm, Cytoplasmic vesicle, Cytoskeleton, Membrane, Microtubule, Mitochondrion.
Database links:	SwissProt: Q9GZQ8 Human | Q9CQV6 Mouse | Q62625 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:100-1:200
IHC-P	1:1,000-1:5,000
IF-Tissue	1:500-1:1,000
IP	Use at an assay dependent concentration.
mIHC	1:100

Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

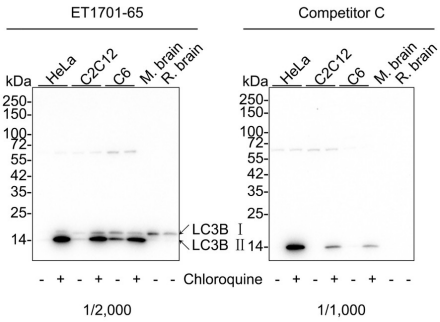

Fig1: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 3: C2C12 cell lysate (20 µg/Lane)
Lane 4: C2C12 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: C6 treated with 50µM Chloroquine for 18 hours cell lysate (20 µg/Lane)
Lane 7: mouse brain tissue lysate (20 µg/Lane)
Lane 8: rat brain tissue lysate (20 µg/Lane)

Predicted band size: 14/16 kDa
Observed band size: 14/16 kDa

Exposure time: 3 minutes;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-65) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

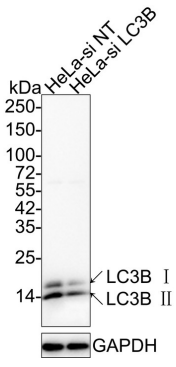

Fig2: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/2,000 dilution.

Lane 1: HeLa-si NT cell lysate (10 µg/Lane)
Lane 2: HeLa-si LC3B cell lysate (10 µg/Lane)

Predicted band size: 14/16 kDa
Observed band size: 14/16 kDa

Exposure time: 1 minute; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-65) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

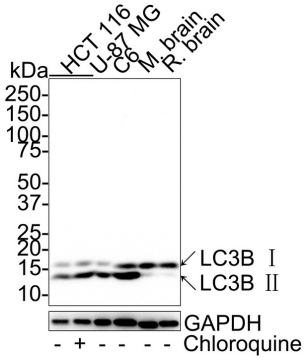

Fig3: Western blot analysis of LC3B on different lysates with Rabbit anti-LC3B antibody (ET1701-65) at 1/2,000 dilution.

Lane 1: HCT 116 cell lysate
Lane 2: HCT 116 treated with 50μM Chloroquine for 18 hours cell lysate
Lane 3: U-87 MG cell lysate
Lane 4: C6 cell lysate
Lane 5: Mouse brain tissue lysate
Lane 6: Rat brain tissue lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 14/16 kDa
Observed band size: 14/16 kDa

Exposure time: 26 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-65) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

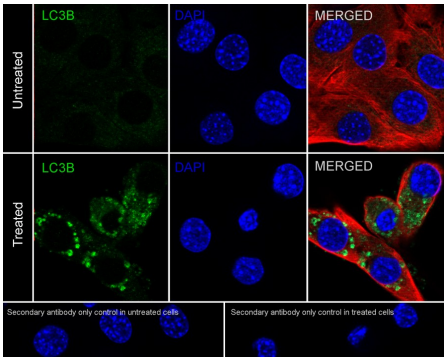

Fig4: Immunocytochemistry analysis of C2C12 cells treated with or without 50μM Chloroquine for 24 hours labeling LC3B with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

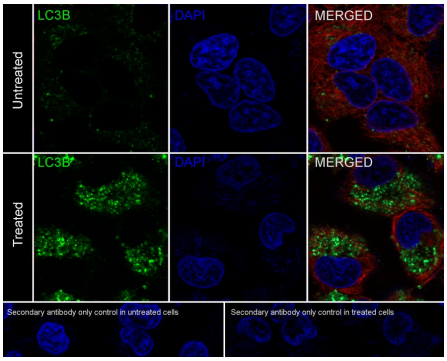

Fig5: Immunocytochemistry analysis of HeLa cells treated with or without 50μM Chloroquine for 24 hours labeling LC3B with Rabbit anti-LC3B antibody (ET1701-65) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-LC3B antibody (ET1701-65) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

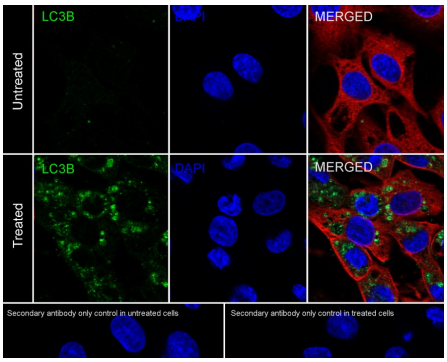

Fig6: Immunocytochemistry analysis of C6 cells treated with or without 50μM Chloroquine for 24 hours labeling LC3B with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-LC3B antibody (ET1701-65) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

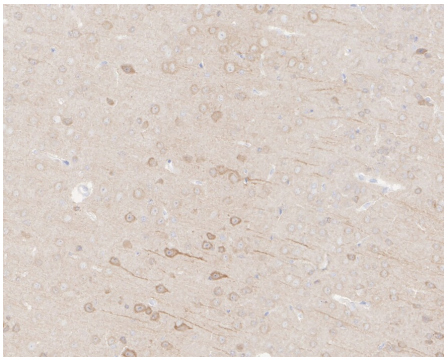

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-LC3B antibody (ET1701-65) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-65) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig8: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-LC3B antibody (ET1701-65) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-65) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

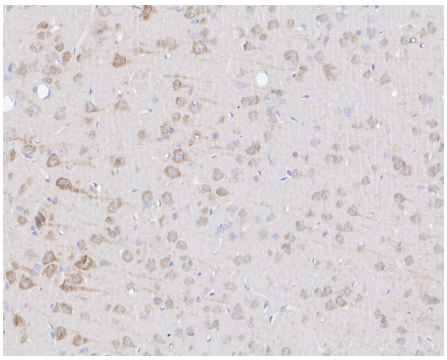

Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-LC3B antibody (ET1701-65) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-65) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

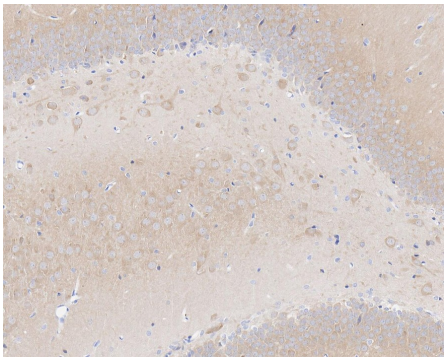

Fig10: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-LC3B antibody (ET1701-65) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-65) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

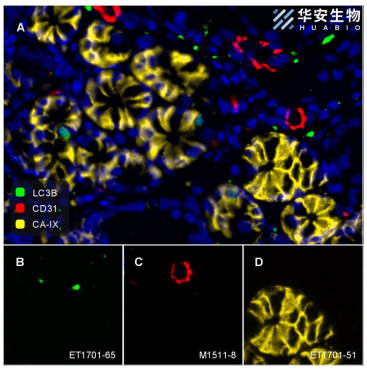

Fig11: Fluorescence multiplex immunohistochemical analysis of human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-LC3B (ET1701-65, Green), anti-CD31 (M1511-8, Red) and anti-CA-IX (ET1701-51, Yellow) on human gastric cancer. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH900205). The section was incubated in three rounds of staining: in the order of ET1701-65 (1/100 dilution), M1511-8 (1/2,000 dilution) and ET1701-51 (1/100 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Background References

1. Omrane M et al. LC3B is lipidated to large lipid droplets during prolonged starvation for noncanonical autophagy. Dev Cell. 2023 Jul
2. Hwang HJ et al. LC3B is an RNA-binding protein to trigger rapid mRNA degradation during autophagy. Nat Commun. 2022 Mar