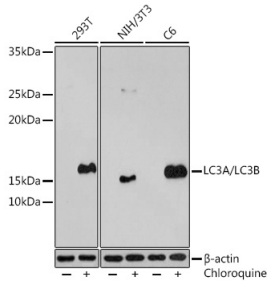
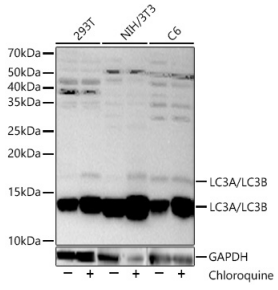
Product Data Sheet:
MAP1LC3A / MAP1LC3B Rabbit Polyclonal Antibody

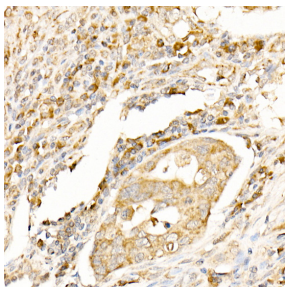
Cat. No:	AB-83557
Conjugate:	Unconjugated
Size:	100 ul
Clone:	POLY
Concentration:	1mg/ml
Host:	Rabbit
Isotype:	IgG
Immunogen:	A synthetic peptide corresponding to a sequence within amino acids 1-60 of human LC3A
Reactivity:	Human,Mouse, Rat
Applications:	Western Blot: 1:500 – 1:2000 Immunohistochemistry(paraffin-embedded tissues): 1:50 – 1:200 Immunofluorescence: Immunocytochemistry: 1:50 – 1:200
Molecular Weight:	14-16kDa
Purification:	Purified
Synonyms:	MAP1LC3B;ATG8F;LC3B;MAP1A/1BLC3;MAP1LC3B-a;MAP1LC3A;ATG8E;LC3;LC3A;MAP1ALC3;MAP1BLC3;LC3A/LC3B
Background:	MAP1A and MAP1B are microtubule-associated proteins which mediate the physical interactions between microtubules and components of the cytoskeleton. MAP1A and MAP1B each consist of a heavy chain subunit and multiple light chain subunits. The protein encoded by this gene is one of the light chain subunits and can associate with either MAP1A or MAP1B. Two transcript variants encoding different isoforms have been found for this gene. The expression of variant 1 is suppressed in many tumor cell lines, suggesting that may be involved in carcinogenesis.
Form:	Liquid
Buffer:	PBS with 0.01% thimerosal,50% glycerol,pH7.3.
Storage:	Store at -20℃. Avoid freeze / thaw cycles.



Western blot analysis of extracts of various cell lines, using LC3A/LC3B antibody at 1:500 dilution.293T cells were treated by Chloroquine (50 μM) at 37℃ for 20 hours.NIH/3T3 cells were treated by Chloroquine (50 μM) at 37℃ for 20 hours.C6 cells were treated by Chloroquine (50 μM) at 37℃ for 20 hours.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) at 1:10000 dilution.
Lysates/proteins: 25μg per lane.
Blocking buffer: 3% BSA.
Detection: ECL West Pico Plus.
Exposure time: 3min.



Western blot analysis of various lysates, using LC3A/LC3B Rabbit pAb at 1:2000 dilution.293T,NIH/3T3 and C6 cells were treated by Chloroquine (50 μM) at 37℃ for 20 hours.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL West Pico Plus.
Exposure time: 30s.



Immunohistochemistry analysis of paraffin-embedded human colon carcinoma using LC3A/LC3B Rabbit pAb at dilution of 1:100 (40x lens).Perform high pressure antigen retrieval with 10 mM citrate buffer pH 6.0 before commencing with IHC staining protocol.

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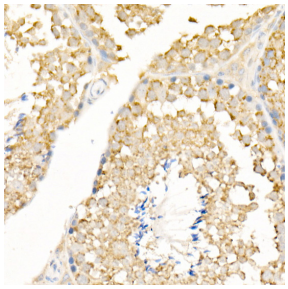
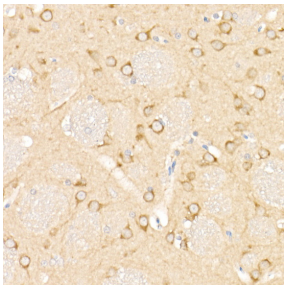
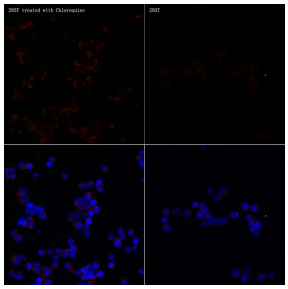
MAP1LC3A / MAP1LC3B Rabbit Polyclonal Antibody



Immunohistochemistry analysis of paraffin-embedded mouse testis using LC3A/LC3B Rabbit pAb at dilution of 1:100 (40x lens).Perform high pressure antigen retrieval with 10 mM citrate buffer pH 6.0 before commencing with IHC staining protocol.



Immunohistochemistry analysis of paraffin-embedded rat brain using LC3A/LC3B Rabbit pAb at dilution of 1:100 (40x lens).Perform high pressure antigen retrieval with 10 mM citrate buffer pH 6.0 before commencing with IHC staining protocol.



Immunofluorescence analysis of 293T Treated with Chloroquine and 293T cells using LC3A/LC3B Rabbit pAb at dilution of 1:100 (40x lens). Blue: DAPI for nuclear staining.

Western Blot Protocol All steps are carried out at room temperature unless otherwise indicated.

A. SDS-PAGE
1. Prepare 15 SDS-PAGE gel.
2. Prepare samples in microfuge tubes. Add 4X SDS sample buffer so the total protein amount is 25 µg per sample
3. Flick microfuge tubes to mix samples, and then heat to 95-100°C for 5 minutes.
4. Set up electrophoresis apparatus and immerse in 1X electrophoretic buffer.
Remove gel combs and cleanse wells of any residual stacking gel.
5. Load samples and protein markers onto the gel using gel loading tips. Set electrophoresis power pack to 80V (through the stacking gel). Increase it to 120V when the protein front reaches the separation gel.

B. Protein Transfer
1. Wet transfers are performed under 200mA constant current for 40min with an ice pack and at 4°C to mitigate the heat produced.
2. Sequentially assemble the transfer constituents and ensure no bubbles lie between any of the layers. Apply wet transfer systems according to the manufacturer's instructions.

C. Immunoblotting 2. After transfer, wash the membrane twice with distilled water, and using a pencil, mark bands of the MW ladder on the membrane.
3. Block with 1X TBST containing 3% nonfat dry milk with constant rocking for 1 hour .
4. Dilute primary antibody in blocking solution with a starting dilution ratio of 1:500.Incubate the membrane with primary antibody overnight at 4°C, on a bench-top rocker.
5. Wash membrane three times with 1X TBST for 10 minutes each.
6. Incubate the membrane with a suitable HRP-conjugated secondary antibody,diluted in 1X TBST. Incubate for 1 hour with constant rocking.
7. Wash membrane three times with 1X TBST for 10 minutes each.

D. Signal Detection
1. Prepare ECL substrate according to the manufacturer's instructions.
2. Incubate the membrane completely with substrate for 20 seconds (adjust time for more sensitive ECL substrates).
3. Expose the membrane to autoradiography film in a dark room or read using a chemiluminescence imaging system.