LC3B Antibody Kit for Autophagy

Catalog no. L10382

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
LC3B, rabbit polyclonal antibody – unconjugated (Component A)	55 µL	1 mg/mL in 10 mM PBS, pH 7.4, 15 mM azide	• ≤–20°C • Protect from light	When stored as directed the product is stable for at least 6 months from the date of receipt.
Chloroquine diphosphate (Component B)	1 mL	30 mM aqueous solution		

Introduction

Autophagy describes the segregation and delivery of cytoplasmic cargo, including proteins and organelles, for degradation by hydrolytic enzymes in "autophagolysosomes", also referred to as "autolysosomes". Although first described in 1963, it has only been in the past decade that this pathway has been the subject of intense research to gain further insight into the role basal autophagy plays in cell homeostasis and development. Efforts are also directed to further elucidate the role of induced autophagy as a cell survival response to stress, microbial infection, and disease (e.g., neurodegeneration, cancer).[1–3]

The LC3B protein plays a critical role in autophagy. Normally, this protein resides in the cytosol, but following cleavage and lipidation with phosphatidylethanolamine, LC3 associates with the phagophore. This localization can be used as a general marker for autophagic membranes (Figure 1).

Each LC3B Antibody Kit for Autophagy includes a rabbit polyclonal antibody against LC3B that has been validated for use in fluorescence microscopy and high content imaging and analysis. The kit also includes chloroquine diphosphate for artificially generating autophagosomes (Figure 2). Following chloroquine diphosphate treatment, lysosomal pH increases and the normal autophagic flux is disrupted, resulting in autophagosome accumulation.

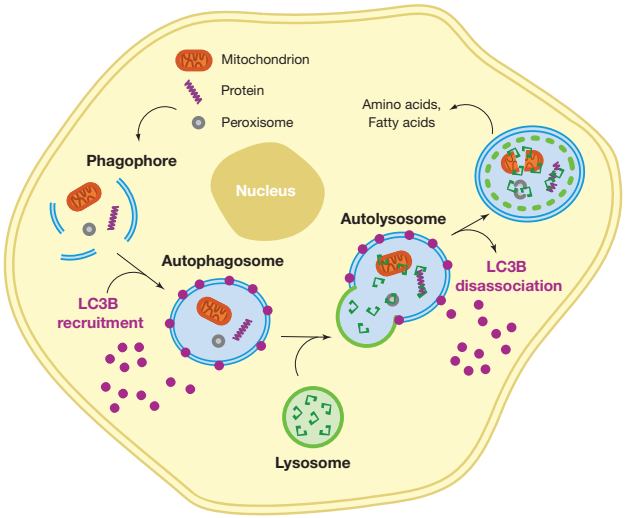

Figure 1. Schematic depiction of the autophagy pathway in a eukaryotic cell. The first step involves the formation and elongation of the isolation membranes or phagophore. The second step entails the expansion and sequestering of the cytoplasm and formation of the double-membrane autophagosome and includes the association of the cytosolic LC3 protein. Fusion of lysosomes with autophagosome to generate the autolysosome is the penultimate step. In the fourth and final phase, the cargo is degraded.

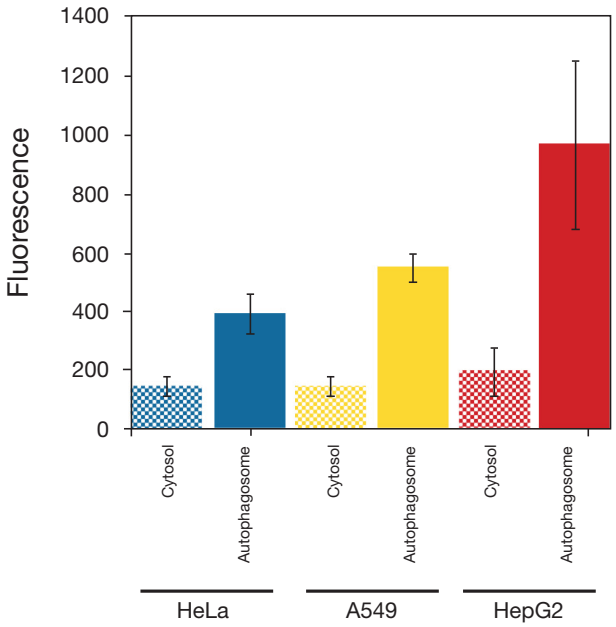

Figure 2. Autophagy detection with the LC3B Antibody Kit. HeLa, A549 and HepG2 cells were treated with 50 µM chloroquine for 16 hours at 37°C. Following fixation and permeabilization, autophagosomes were stained with LC3B rabbit polyclonal antibody and visualized with Alexa Fluor® 647 goat anti-rabbit IgG. The fluorescence intensity of the autophagosomes and the cytosol were quantified using Slidebook™ digital microscopy software.

Before Starting

Materials Required but Not Provided

- Phosphate buffered saline (PBS), pH 7.2–7.6
- Fixative (i.e., 3.7% formaldehyde in PBS)
- Permeabilization reagent (i.e., 0.2% Triton® X-100 in PBS)
- Blocking buffer
- Anti-rabbit secondary detection reagent

Experimental Protocols

The following protocol was optimized for fluorescence microscopy and high-content imaging and analysis using cells fixed with 3.7% formaldehyde in PBS and permeabilized with 0.2% Triton® X-100 in PBS, but the LC3B antibody is also amenable to other fixation/permeabilization reagents.

1.1 *Optional*: Treat cells with 30–100 µM chloroquine (Component B) for 12–16 hours.

1.2 Dilute the LC3B rabbit polyclonal antibody in blocking buffer to prepare 0.5 µg/mL working solution. Note that this final concentration may require further optimization.

1.3 Add 3.7% formaldehyde in PBS to the cells and incubate for 15 minutes at room temperature.

1.4 Remove the fixative and wash the cells three times with PBS.

1.5 Remove the wash solution, add 0.2% Triton® X-100 in PBS to the cells, and incubate them for 15 minutes at room temperature.

1.6 Remove the permeabilization buffer, add the diluted primary antibody (prepared in Step 1.2) to the cells, and incubate them for 1 hour at room temperature.

1.7 Remove the diluted primary antibody and wash the cells three times with PBS.

1.8 Remove the wash solution and incubate the cells with an anti-rabbit secondary antibody.

1.9 Wash the cells with PBS and perform any additional staining (i.e., DNA counterstain, other antibodies).

1.10 Image the cells using the appropriate filters for the anti-rabbit secondary detection reagent. Autophagosomes are vesicular structures typically located in the perinuclear region.

References

1. Molecular Cell Biology 8, 931 (2007); **2.** Genes & Development 21, 2861 (2007); **3.** Drug Discovery 6, 304 (2007).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
L10382	LC3B Antibody Kit for Autophagy *rabbit polyclonal LC3B* *includes autophagosome inducer*	1 kit
Related Products		
P36235	Premo™ Autophagy Sensor LC3B-GFP	1 kit
P36236	Premo™ Autophagy Sensor LC3B-RFP	1 kit
A11008	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11012	Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11034	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11037	Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11039	Alexa Fluor® 350 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11046	Alexa Fluor® 350 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21068	Alexa Fluor® 350 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21206	Alexa Fluor® 488 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21207	Alexa Fluor® 594 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21244	Alexa Fluor® 647 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21245	Alexa Fluor® 647 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21248	Alexa Fluor® 555 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21249	Alexa Fluor® 555 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21441	Alexa Fluor® 488 chicken anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21442	Alexa Fluor® 594 chicken anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21443	Alexa Fluor® 647 chicken anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A31572	Alexa Fluor® 555 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A31573	Alexa Fluor® 647 donkey anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL

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