

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat LC3B in Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1251B
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human LC3B synthetic peptide Accession # Q9H492
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

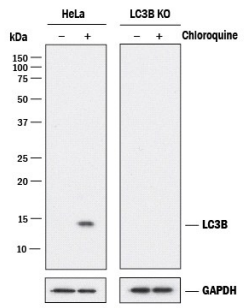
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Knockout Validated	0.1 µg/mL	See Below
Western Blot	0.1 µg/mL	See Below
Immunocytochemistry	1-25 µg/mL	See Below
Simple Western	5 µg/mL	See Below

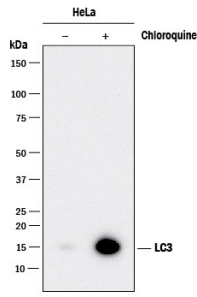
DATA

Knockout Validated



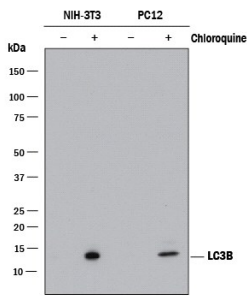
Western Blot Show Human LC3A Specificity Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and LC3B knockout HeLa cell line (KO) untreated (-) or treated (+) with 50µM Chloroquine for 18 hours. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human LC3A Monoclonal Antibody (Catalog # MAB85582) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for LC3A at approximately 15 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



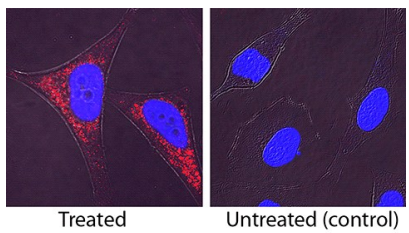
Detection of Human LC3B by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 50µM Chloroquine for 18 Hours. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human LC3B Monoclonal Antibody (Catalog # MAB85582) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for LC3B at approximately 15 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



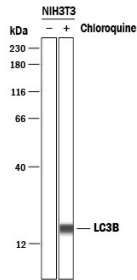
Detection of Mouse and Rat LC3A by Western Blot. Western blot shows lysates of NIH-3T3 mouse embryonic fibroblast cell line and PC-12 rat adrenal pheochromocytoma cell line untreated (-) or treated (+) with 50µM Chloroquine for 18 hours. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human LC3A Monoclonal Antibody (Catalog # MAB85582) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for LC3A at approximately 15 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



LC3B in HeLa Human Cell Line. LC3B was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with Chloroquine using Rabbit Anti-Human LC3B Monoclonal Antibody (Catalog # MAB85582) at 1 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Simple Western



Detection of Mouse LC3A by Simple Western™. Simple Western lane view shows lysates of NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 50uM Chloroquine for 18 hours, loaded at 0.2 mg/mL. A specific band was detected for LC3A at approximately 17 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human LC3A Monoclonal Antibody (Catalog # MAB85582). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Human Microtubule-associated Protein (MAP) Light Chain 3 (LC3) A is a 121 amino acid (aa) protein with a predicted molecular weight of 14 kDa. It is a member of the LC3 subfamily of Autophagy-related 8 (Atg8) proteins (1). The LC3 subfamily also includes LC3B and LC3C. LC3 exhibits 100% aa sequence identity with its mouse and rat orthologs, and is orthologous to the yeast autophagy-related protein Atg8. Atg8 family members show structural similarity with Ubiquitin, but lack aa sequence similarity. LC3 was originally described as part of a complex that includes heavy and light chains comprising the MAP1 family of microtubule regulatory proteins (3). However, LC3 has gained attention for MAP1-independent functions in autophagy. LC3 utilizes a ubiquitin-like conjugation system that includes E1-, E2-, and E3-like enzymes to covalently attach phosphatidylethanolamine (PE) to its C-terminus, incorporating it into the phagophore membrane during the early stages of autophagosome formation (4). Recruitment of LC3 to the phagophore may promote membrane elongation (4,5). It may also be involved in cargo recruitment to autophagosomes (1). LC3 is often used as a marker of autophagy.

References:

1. Shpilka, T. *et al.* (2011) *Genome Biol.* **12**:226.
2. He, H. *et al.* (2003) *J. Biol. Chem.* **278**:29278.
3. Kuznetsov, S.A. & V.I. Gelfand (1987) *FEBS Let.* **212**:145.
4. Weidberg, H. *et al.* (2011) *Ann Rev. Biochem.* **80**:125.
5. Weidberg, H. *et al.* (2010) *EMBO J.* **29**:1792.