

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti- LC3 produced in rabbit, affinity isolated antibody

Catalog Number L8918

Product Description

Anti- LC3 is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 36-49 of human LC3A isoform a (GeneID: 84557) and LC3B (GeneID: 81631), conjugated to KLH via a C-terminal cysteine residue. The corresponding sequence is identical in rat and mouse. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti- LC3 recognizes human and rat LC3A/B-I and LC3A/B-II by immunoblotting and immunoprecipitation (~18 kDa and ~16 kDa, respectively). Anti- LC3 recognizes mouse LC3A/B-I (~18 kDa). The detection of mouse LC3A/B-II may require specific cell treatment and/or long exposure. Detection of the LC3A/B bands by immunoblotting is specifically inhibited with the immunizing peptide.

Macroautophagy, usually referred to as autophagy, is a major pathway for bulk degradation of cytoplasmic constituents and organelles. In this process, portions of the cytoplasm are sequestered into double membrane vesicles, the autophagosomes, and subsequently delivered to the lysosome for degradation and recycling. 1,2 Although autophagy is a constitutive cellular event, it is enhanced under certain conditions such as starvation, hormonal stimulation and drug treatments.³ Autophagy is required for normal turnover of cellular components during starvation. It plays an essential role in cellular differentiation, cell death and aging. Defective autophagy may contribute to certain human diseases such as cancer, neurodegenerative diseases, muscular disorders and pathogen infections. 4,5 Autophagy is an evolutionarily conserved pathway seen in all eukaryotic cells.

At least 16 genes encoding for autophagy (ATG) related proteins that are required for autophagosome formation were identified in yeast by genetic screens. For many of these genes, related homologs have been identified in mammals.⁶

Microtubule-associated protein light chain 3 (LC3), the mammalian homolog of yeast Atg8/Apg8/Aut7, is essential for the formation of autophagosomes. LC3 was first identified in rat as a protein that co-purifies with microtubule-associated protein 1A and 1B from rat brain.3 LC3 exists in cells in two forms. One is cytoplasmic, LC3-I (18 kDa) and the other, LC3-II (16 kDa) is associated with the autophagosome membrane. Following synthesis, the carboxy-terminal region of proLC3 is cleaved by the cysteine protease Atg4, generating the soluble LC3-I and exposing a carboxy- terminal Gly¹²⁰. LC3-I is modified to a membrane-bound form, LC3-II (a LC3-phospholipid conjugate), by mammalian Atg7 and Atg3, which are E1- and E2-like enzymes, respectively. The amount of LC3 II correlates with the extent of autophagosome formation.³ Three human orthologs of yeast Atg8. named MAP1LC3A/LC3A, MAP1LC3B/LC3B and MAP1LC3C/LC3C, were identified. The human proteins exhibit two forms representing the cytosolic (type I) and the membrane associated (type II) forms. The three human isoforms show different expression patterns in human tissues.8

LC3 can be used as an autophagosomal marker. 3,7,9

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as preservative.

Antibody concentration: ~ 1.0 mg/ml

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 1-2 μ g/ml is recommended using whole extracts of rat and mouse brain.

Immunoprecipitation: a working amount of 5-10 µg is recommended using extracts of human U87 cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

- Klionsky, D.J., and Emr, S.D., Science, 290, 1717-1721 (2000).
- Kuma, A., et al., Nature, 432, 1032-1036 (2004).
- 3. Kabeya, Y., et al., *EMBO J.*, **19**, 5720-5728 (2000).
- 4. Reggiori, F., and Klionsky, D.J., *Eukaryotic Cell*, **1**, 11-21 (2002).
- Shintani, T., and Klionsky, D.J., Science, 306, 990-995 (2004).
- Klionsky, D.J., et al., *Develop. Cell*, 5, 539-545 (2003).
- 7. Tanida, I., et al., *J. Biol. Chem.*, **279**, 47704-47710 (2004).
- 8. He, H., et al., *J. Biol. Chem.*, **278**, 29278-29287 (2003).
- 9. Wu, J., et al., *Biochem. Biophys. Res. Commun.*, **339**, 437-442 (2006).

ST,KAA,PHC 09/07-1