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ProductInformation

Anti-LC3B
produced in rabbit, affinity isolated antibody

Catalog Number **L7543**

Product Description

Anti-LC3B is developed in rabbit using as immunogen a synthetic peptide corresponding to amino acids 2-15 of human LC3B (Gene ID: 81631), conjugated to KLH via a C-terminal cysteine residue. The corresponding sequence differs by one amino acid in rat and mouse. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-LC3B recognizes human, rat, and mouse LC3B-I and LC3B-II by immunoblotting (~18 kDa and ~16 kDa, respectively) and by immunofluorescence. A higher molecular weight band may be seen in some mouse brain preparations. Detection of the LC3B 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 evolutionary conserved pathway seen in all eukaryotic cells.[1]

At least 16 genes encoding for autopahgy (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]

Rat microtubule-associated protein light chain 3 (LC3), the mammalian homolog of yeast Atg8/Apg8/Aut7, is essential in the formation of autophagosomes.[7] 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 carboxyl terminal region of proLC3 is cleaved by the cysteine protease Atg4, generating the soluble LC3-I and exposing a carboxyl terminal Gly^{120}. 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.[7] The amount of LC3 II correlates with the extent of autophagosome formation.[3] Three human orthologs of the rat LC3, 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]

LC3B can be used as a autophagosomal marker.[3, 7]

Reagent

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

Antibody concentration: ~1 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, or storage in “frost-free” freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

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

Indirect immunofluorescence: a working concentration of 5-10 µg/mL is recommended by staining human HeLa cells.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

1. Klionsky, D.J., and Emr, S.D., *Science*, **290**, 1717-1721 (2000).
2. 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).
5. Shintani, T., and Klionsky, D.J., *Science*, **306**, 990-995 (2004).
6. 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).

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