

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-Atg5 (N-terminal)

produced in rabbit, affinity isolated antibody

Catalog Number A0856

## **Product Description**

Anti-Atg5 (N-terminal) is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 2-15 of human Atg5 (GeneID: 9474), 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-Atg5 (N-terminal) recognizes human, rat, and mouse Atg5-Atg12 complex by immunoblotting (~56 kDa). Detection of the Atg5-Atg12 complex band by immunoblotting is specifically inhibited with the immunizing peptide. An additional band of ~40 kDa may be detected in some cell extracts.

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.<sup>1, 2</sup> Although autophagy is a constitutive cellular event, it is enhanced under certain conditions such as starvation, hormonal stimulation and drug treatments.<sup>3</sup> 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. At least 16 ATG genes required for autophagosome formation were identified in yeast by genetic screens. For many of these genes, related homologs have been identified in mammals.6

Atg5 (also known as Apg5) is a 32 kDa protein essential for autophagy. Atg5 is covalently modified by Atg12, a ubiquitin-like modifier. This conjugation reaction requires ATP and two enzymes, Atg7 and Atg10, which are E1- and E2-like enzymes, respectively. The Atg12-Atg5 conjugate interacts noncovalently with Atg16. The Atg12-Atg5-Atg16 complex

localizes to autophagosome precursors and plays an essential role in autophagosome formation.<sup>7,8</sup> It has been reported that in addition to the role of Atg5 in the formation of autophagosomes, an Atg5 fragment produced by calpain cleavage has pro-apoptotic properties.<sup>9,10</sup>

## 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 0.5-1  $\mu$ g/mL is recommended using whole extracts of human K562, rat NRK, and mouse 3T3 cells.

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

### 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).

- 5. Shintani, T., and Klionsky, D.J., *Science*, **306**, 990-995 (2004).
- 6. Klionsky, Ď.J., et al., *Develop. Cell*, **5**, 539-545 (2003).
- 7. Mizushima, N., et al., *J. Cell Biol.*, **152**, 657-667 (2001).
- 8. Mizushima, N., et al., *J. Cell Sci.*, **116**, 1679-1688 (2003).
- 9. Yousefi, S., et al., *Nat. Cell Biol.*, **8**, 1124 1132 (2006).
- 10. Codogno, P. and Meijer, A.J., *Nat. Cell Biol.*, **8**, 1045-1047 (2006).

ST,KAA,PHC 05/07-1