

## Product Information

### Anti-ATG4A

produced in rabbit, affinity isolated antibody

Catalog Number **A3484**

#### Product Description

Anti-ATG4A is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 100-114 of human ATG4A (GeneID: 115201), conjugated to KLH. The corresponding sequence differs by 2 amino acids in mouse and rat. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-ATG4A recognizes human ATG4A. The antibody can be used for immunoprecipitation (~ 45 kDa).

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.<sup>4,5</sup> Autophagy is an evolutionarily conserved pathway seen in all eukaryotic cells.<sup>1</sup>

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.<sup>6</sup> Two ubiquitin-like conjugation systems are involved in autophagosome formation: Atg12 and Atg8 conjugation systems. Atg8 is synthesized as a precursor protein, which is cleaved after a Gly residue by the cysteine proteinase Atg4. The modified Atg8 is activated by Atg7, an E1-like enzyme, and then transferred to Atg3, an E2-like enzyme, followed by conjugation to membrane-bound phosphatidylethanolamine (PE). The complex Atg8-PE is also deconjugated by Atg4, leading to the release of Atg8 from membranes.<sup>7,8</sup>

Four human orthologues of yeast Atg4 have been identified: HsAtg4A/autophagin-2, HsAtg4B/autophagin-1, HsAtg4C/autophagin-3 and HsAtg4D/autophagin-4.<sup>9</sup> Atg4A cleaves mainly the Atg8 homologue, GATE-16. GATE-16 is cleaved downstream of a conserved and essential Gly<sup>116</sup> *in vivo* and *in vitro*. Atg4A is active as a cysteine protease of GATE-16 only in the presence of the reducing agent DTT. It is regulated by reactive oxygen species (ROS), specifically H<sub>2</sub>O<sub>2</sub>, which is essential for autophagy. Cys<sup>81</sup>, which is located near the catalytic site of Atg4A, is critical for this redox regulation.<sup>10-11</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: ~ 1.0 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

Store at -20 °C. For continuous use, the product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at -20 °C. 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

**Immunoprecipitation:** a working antibody amount of 5-10 µg is recommended using a lysate of HEK-293T cells.

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

## References

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