Revision 1

Store at -20C

#5299

Atg4B Antibody

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W	H M R	Endogenous	48	Rabbit	#Q9Y4P1	23192

Product Usage Information

Application	Dilution
Western Blotting	1:1000

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Atg4B Antibody detects endogenous levels of total Atg4B protein. This antibody detects a band at ~27 kDa of unknown origin.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser372 of human Atg4B protein. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents. Control of autophagy was largely discovered in yeast and involves proteins encoded by a set of autophagy-related genes (Atg) (1). Formation of autophagic vesicles requires a pair of essential ubiquitin-like conjugation systems, Atg12-Atg5 and Atg8-phosphatidylethanolamine (Atg8-PE), which are widely conserved in eukaryotes (2). Numerous mammalian counterparts to yeast Atg proteins have been described, including three Atg8 proteins (GATE-16, GABARAP, and LC3) and four Atg4 homologs (Atg4A/autophagin-2, Atg4B/autophagin-1, Atg4C/autophagin-3, and Atg4D/autophagin-4) (3-5). The cysteine protease Atg4 is pivotal to autophagosome membrane generation and regulation. Atg4 primes the Atg8 homolog for lipidation by cleaving its carboxy terminus and exposing its glycine residue for E1-like enzyme Atg7. The Atg8 homolog is transferred to the E2-like enzyme Atg3 before forming the Atg8-PE conjugate. During later stages of autophagy, Atg4 can reverse this lipidation event by cleaving PE, thereby recycling the Atg8 homolog (6).
While Atg4B displays a broad specificity for Atg8 homologues, it preferentially cleaves LC3 (7-9). Mutation in the corresponding Atg4B gene can be associated with strong inhibition of autophagosome formation. An excess of inactive Atg4B blocks lipidation of Atg8 homologues and inhibits autophagy. This makes Atg4B a potential tool for characterization of the isolation membrane and other autophagy studies (10,11).

Background References

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2. Ohsumi, Y. (2001) *Nat Rev Mol Cell Biol* 2, 211-6.
3. Kabeya, Y. et al. (2000) *EMBO J* 19, 5720-8.
4. Kabeya, Y. et al. (2004) *J Cell Sci* 117, 2805-12.
5. Mariño, G. et al. (2003) *J Biol Chem* 278, 3671-8.
6. Sou, Y.S. et al. (2008) *Mol Biol Cell* 19, 4762-75.
7. Hemelaar, J. et al. (2003) *J Biol Chem* 278, 51841-50.
8. Kabeya, Y. et al. (2004) *J Cell Sci* 117, 2805-12.
9. Tanida, I. et al. (2004) *J Biol Chem* 279, 36268-76.
10. Fujita, N. et al. (2008) *Mol Biol Cell* 19, 4651-9.
11. Fujita, N. et al. (2009) *Autophagy* 5, 88-9.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat

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