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ATG4B Antibody (C-term)

Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP1809c

Product Information

Application	IF, WB, IHC-P, E
Primary Accession	Q9Y4P1
Other Accession	Q8BGE6
Reactivity	Human
Predicted	Mouse, Rat, Bovine, Canine, Rabbit, Chicken
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Calculated MW	44294
Antigen Region	358-390

Additional Information

Gene ID	23192
Other Names	Cysteine protease ATG4B, 3422-, AUT-like 1 cysteine endopeptidase, Autophagin-1, Autophagy-related cysteine endopeptidase 1, Autophagy-related protein 4 homolog B, hAPG4B, ATG4B, APG4B, AUTL1, KIAA0943
Target/Specificity	This ATG4B antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 358-390 amino acids from the C-terminal region of human ATG4B.
Dilution	IF~~1:100 WB~~1:1000 IHC-P~~1:100~500 E~~Use at an assay dependent concentration.
Format	Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is prepared by Saturated Ammonium Sulfate (SAS) precipitation followed by dialysis against PBS.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Precautions	ATG4B Antibody (C-term) is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	ATG4B {ECO:0000303|PubMed:15187094, ECO:0000312|HGNC:HGNC:20790}

Function	Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins (PubMed:15169837, PubMed:15187094, PubMed:17347651, PubMed:19322194, PubMed:21177865, PubMed:22302004, PubMed:26378241, PubMed:27527864, PubMed:28633005, PubMed:28821708, PubMed:29232556, PubMed:30076329, PubMed:30443548, PubMed:30661429). Required for canonical autophagy (macroautophagy), non-canonical autophagy as well as for mitophagy (PubMed:33773106, PubMed:33909989). The protease activity is required for proteolytic activation of ATG8 family proteins: cleaves the C-terminal amino acid of ATG8 proteins MAP1LC3A, MAP1LC3B, MAP1LC3C, GABARAPL1, GABARAPL2 and GABARAP, to reveal a C- terminal glycine (PubMed:15169837, PubMed:15187094, PubMed:17347651, PubMed:19322194, PubMed:20818167, PubMed:21177865, PubMed:22302004, PubMed:27527864, PubMed:28287329, PubMed:28633005, PubMed:29458288, PubMed:30661429). Exposure of the glycine at the C-terminus is essential for ATG8 proteins conjugation to phosphatidylethanolamine (PE) and insertion to membranes, which is necessary for autophagy (PubMed:15169837, PubMed:15187094, PubMed:17347651, PubMed:19322194, PubMed:21177865, PubMed:22302004). Protease activity is also required to counteract formation of high-molecular weight conjugates of ATG8 proteins (ATG8ylation): acts as a deubiquitinating-like enzyme that removes ATG8 conjugated to other proteins, such as ATG3 (PubMed:31315929, PubMed:33773106). In addition to the protease activity, also mediates delipidation of ATG8 family proteins (PubMed:15187094, PubMed:19322194, PubMed:28633005, PubMed:29458288, PubMed:32686895, PubMed:33909989). Catalyzes delipidation of PE- conjugated forms of ATG8 proteins during macroautophagy (PubMed:15187094, PubMed:19322194, PubMed:29458288, PubMed:32686895, PubMed:33909989). Also involved in non-canonical autophagy, a parallel pathway involving conjugation of ATG8 proteins to single membranes at endolysosomal compartments, by catalyzing delipidation of ATG8 proteins conjugated to phosphatidylserine (PS) (PubMed:33909989). Compared to other members of the family (ATG4A, ATG4C or ATG4C), constitutes the major protein for proteolytic activation of ATG8 proteins, while it displays weaker delipidation activity than other ATG4 paralogs (PubMed:29458288, PubMed:30661429). Involved in phagophore growth during mitophagy independently of its protease activity and of ATG8 proteins: acts by regulating ATG9A trafficking to mitochondria and promoting phagophore-endoplasmic reticulum contacts during the lipid transfer phase of mitophagy (PubMed:33773106).
Cellular Location	Cytoplasm. Cytoplasm, cytosol. Cytoplasmic vesicle, autophagosome. Endoplasmic reticulum. Mitochondrion. Note=Mainly localizes to the cytoplasm, including cytosol (PubMed:29165041). A samll potion localizes to mitochondria; phosphorylation at Ser-34 promotes localization to mitochondria (PubMed:29165041).

Background

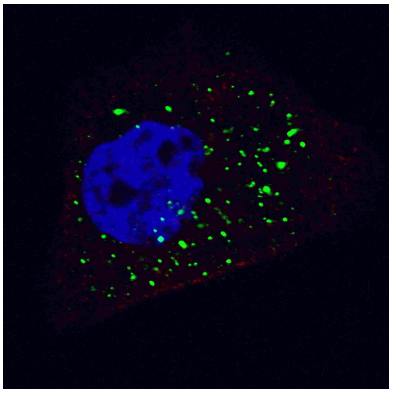
Macroautophagy is the major inducible pathway for the general turnover of cytoplasmic constituents in eukaryotic cells, it is also responsible for the degradation of active cytoplasmic enzymes and organelles during nutrient starvation. Macroautophagy involves the formation of double-membrane bound autophagosomes which enclose the cytoplasmic constituent targeted for degradation in a membrane bound structure, which then fuse with the lysosome (or vacuole) releasing a single-membrane bound autophagic bodies which are then degraded within the lysosome (or vacuole). APG4 is a cysteine protease required for autophagy, which cleaves the C-terminal part of either MAP1LC3, GABARAPL2 or GABARAP, allowing the liberation of form I. A subpopulation of form I is subsequently converted to a smaller form (form II). Form II, with a revealed C-terminal glycine, is considered to be the phosphatidylethanolamine (PE)-conjugated form,

and has the capacity for the binding to autophagosomes.

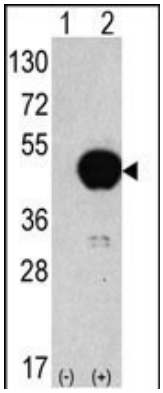
References

References for protein:
1.Baehrecke EH. Nat Rev Mol Cell Biol. 6(6):505-10. (2005)
2. Lum JJ, et al. Nat Rev Mol Cell Biol. 6(6):439-48. (2005)
3.Greenberg JT. Dev Cell. 8(6):799-801. (2005)
4.Levine B. Cell. 120(2):159-62. (2005)
5.Shintani T and Klionsky DJ. Science. 306(5698):990-5. (2004)
References for U251 cell line:
1. Westermark B.; Pontén J.; Hugosson R. (1973).” Determinants for the establishment of permanent tissue culture lines from human gliomas”. Acta Pathol Microbiol Scand A. 81:791-805. [PMID: 4359449].
2. Pontén, J.,Westermark B. (1978).” Properties of Human Malignant Glioma Cells in Vitro”. Medical Biology 56: 184-193.[PMID: 359950].
3. Geng Y.;Kohli L.; Klocke B.J.; Roth K.A.(2010). “Chloroquine-induced autophagic vacuole accumulation and cell death in glioma cells is p53 independent”. Neuro Oncol. 12(5): 473–481.[PMID: 20406898].

Images

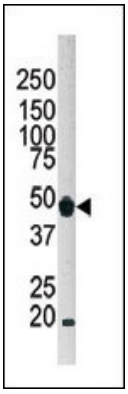


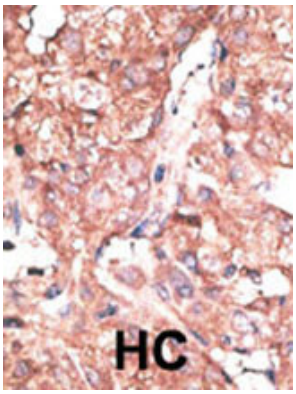
Fluorescent image of U251 cells stained with ATG4B (C-term) antibody. U251 cells were treated with Chloroquine (50 µM,16h), then fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP1809c ATG4B (C-term) primary antibody (1:100, 2 h at room temperature). For secondary antibody, Alexa Fluor® 488 conjugated donkey anti-rabbit antibody (green) was used (1:1000, 1h). Nuclei were counterstained with Hoechst 33342 (blue) (10 µg/ml, 5 min). ATG4B immunoreactivity is localized to autophagic vacuoles in the cytoplasm of U251 cells.



Western blot analysis of anti-hAPG4B-373 Pab (Cat. #AP1809c) in 293 cell line lysates transiently transfected with the ATG4B gene (2ug/lane). hAPG4B-373(arrow) was detected using the purified Pab.

Western blot analysis of anti-APG4B Pab (Cat. #AP1809c) in Hela cell lysate. APG4B (arrow) was detected using the purified Pab.





Formalin-fixed and paraffin-embedded human cancer tissue reacted with the primary antibody, which was peroxidase-conjugated to the secondary antibody, followed by DAB staining. This data demonstrates the use of this antibody for immunohistochemistry; clinical relevance has not been evaluated. BC = breast carcinoma; HC = hepatocarcinoma.

Citations

- A high-throughput FRET-based assay for determination of Atg4 activity.
- Induction of an incomplete autophagic response by cancer-preventive geranylgeranoic acid (GGA) in a human hepatoma-derived cell line.
- Kinetics comparisons of mammalian Atg4 homologues indicate selective preferences toward diverse Atg8 substrates.

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