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ATG9A Antibody (C-term)

Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP1814C

Product Information

Application	IF, WB, IHC-P, E
Primary Accession	Q7Z3C6
Reactivity	Human
Predicted	Mouse, Rat, Bovine, Canine, Rabbit
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Calculated MW	94447
Antigen Region	717-746

Additional Information

Gene ID	79065
Other Names	Autophagy-related protein 9A, APG9-like 1, mATG9, ATG9A, APG9L1
Target/Specificity	This ATG9A antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 717-746 amino acids from the C-terminal region of human ATG9A.
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	ATG9A Antibody (C-term) is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	ATG9A {ECO:0000303|PubMed:20124090, ECO:0000312|HGNC:HGNC:22408}
Function	Phospholipid scramblase involved in autophagy by mediating autophagosomal membrane expansion (PubMed:22456507, PubMed:27510922, PubMed:29437695, PubMed:32513819,

	PubMed:32610138, PubMed:33106659, PubMed:33468622, PubMed:33850023). Cycles between the preautophagosomal structure/phagophore assembly site (PAS) and the cytoplasmic vesicle pool and supplies membrane for the growing autophagosome (PubMed:16940348, PubMed:22456507, PubMed:33106659). Lipid scramblase activity plays a key role in preautophagosomal structure/phagophore assembly by distributing the phospholipids that arrive through ATG2 (ATG2A or ATG2B) from the cytoplasmic to the luminal leaflet of the bilayer, thereby driving autophagosomal membrane expansion (PubMed:33106659). Also required to supply phosphatidylinositol 4- phosphate to the autophagosome initiation site by recruiting the phosphatidylinositol 4-kinase beta (PI4KB) in a process dependent on ARFIP2, but not ARFIP1 (PubMed:30917996). In addition to autophagy, also plays a role in necrotic cell death (By similarity).
Cellular Location	Preautophagosomal structure membrane; Multi-pass membrane protein. Cytoplasmic vesicle, autophagosome membrane; Multi- pass membrane protein. Golgi apparatus, trans-Golgi network membrane; Multi-pass membrane protein. Late endosome membrane; Multi-pass membrane protein. Recycling endosome membrane; Multi-pass membrane protein. Endoplasmic reticulum membrane; Multi-pass membrane protein. Mitochondrion membrane; Multi-pass membrane protein. Note=Mainly localizes to the trans-Golgi network (TGN) and the endosomal system; cycles between them though vesicle trafficking (PubMed:27316455, PubMed:27663665). Export from the TGN to promote formation of autophagosomes is mediated by the AP-4 complex (PubMed:29180427, PubMed:30262884). Under amino acid starvation or rapamycin treatment, redistributes to preautophagosomal structure/phagophore assembly site (PAS) (PubMed:16940348). The starvation-induced redistribution depends on ULK1, ATG13, as well as SH3GLB1 (PubMed:16940348). Upon autophagy induction, a small portion transiently localizes to the autophagic membranes (PubMed:22456507) Recruited to damaged mitochondria during mitophagy in a RIMOC1- dependent manner (PubMed:34432599).

Background

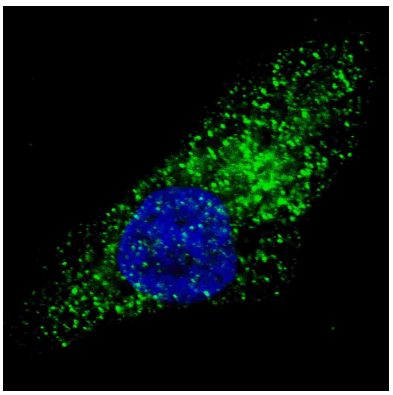
Macroautophagy is the major inducible pathway for the general turnover of cytoplasmic constituents in eukaryotic cells, it is also responsible for the degradation of active cytoplasmic enzymes and organelles during nutrient starvation. Macroautophagy involves the formation of double-membrane bound autophagosomes 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). Apg9 plays a direct role in the formation of the cytoplasm to vacuole targeting and autophagic vesicles, possibly serving as a marker for a specialized compartment essential for these vesicle-mediated alternative targeting pathways.

References

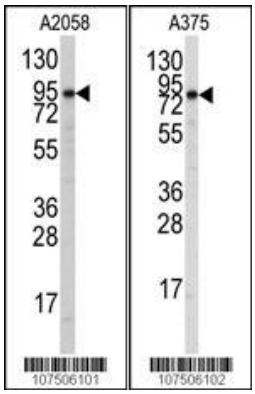
References for protein:
1.Baehrecke EH. Nat Rev Mol Cell Biol. 6(6):505-10. (2005)
2.Lum JJ, et al. Nat Rev Mol Cell Biol. 6(6):439-48. (2005)
3.Greenberg JT. Dev Cell. 8(6):799-801. (2005)
4.Levine B. Cell. 120(2):159-62. (2005)
5.Shintani T and Klionsky DJ. Science. 306(5698):990-5. (2004)
References 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].

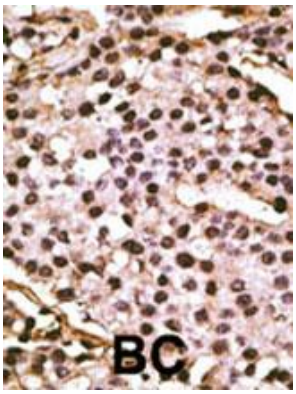
Images



Fluorescent image of U251 cells stained with ATG9A (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 AP1814c ATG9A (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). ATG9A immunoreactivity is localized to autophagic vacuoles in the cytoplasm of U251 cells.



Western blot analysis of anti-Autophagy APG9L1 Antibody (C-term) (Cat.#AP1814c) in A2058 and A375 cell line lysates (35ug/lane). APG9L1(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

- Therapeutic potential of a synthetic lethal interaction between the MYC proto-oncogene and inhibition of aurora-B kinase.
- Biochemical isolation and characterization of the tubulovesicular LC3-positive autophagosomal compartment.

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