

#### ATG5 Antibody (N-term)

Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP1812a

# **Specification**

## ATG5 Antibody (N-term) - Product Information

Application WB, IF, IHC-P-Leica, E

Primary Accession <u>Q9H1Y0</u>

Other Accession <u>Q3MQ06</u>, <u>Q3MQ04</u>, <u>Q99|83</u>, <u>Q3MQ24</u>, <u>Q6DEM4</u>

Reactivity Human, Mouse Predicted Bovine, Pig, Rat

Host Rabbit
Clonality Polyclonal
Isotype Rabbit IgG

Antigen Region 1-30

### ATG5 Antibody (N-term) - Additional Information

#### **Gene ID 9474**

#### **Other Names**

Autophagy protein 5, APG5-like, Apoptosis-specific protein, ATG5, APG5L, ASP

#### Target/Specificity

This ATG5 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 1-30 amino acids from the N-terminal region of human ATG5.

#### **Dilution**

WB~~1:1000 IF~~1:200

IHC-P-Leica~~1:500

E~~Use at an assay dependent concentration.

#### **Format**

Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.

# **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**

ATG5 Antibody (N-term) is for research use only and not for use in diagnostic or therapeutic procedures.

### ATG5 Antibody (N-term) - Protein Information

Name ATG5 (HGNC:589)



# Synonyms APG5L, ASP

**Function** Involved in autophagic vesicle formation. Conjugation with ATG12, through a ubiquitin-like conjugating system involving ATG7 as an E1-like activating enzyme and ATG10 as an E2-like conjugating enzyme, is essential for its function. The ATG12-ATG5 conjugate acts as an E3-like enzyme which is required for lipidation of ATG8 family proteins and their association to the vesicle membranes. Involved in mitochondrial quality control after oxidative damage, and in subsequent cellular longevity. Plays a critical role in multiple aspects of lymphocyte development and is essential for both B and T lymphocyte survival and proliferation. Required for optimal processing and presentation of antigens for MHC II. Involved in the maintenance of axon morphology and membrane structures, as well as in normal adipocyte differentiation. Promotes primary ciliogenesis through removal of OFD1 from centriolar satellites and degradation of IFT20 via the autophagic pathway. As part of the ATG8 conjugation system with ATG12 and ATG16L1, required for recruitment of LRRK2 to stressed lysosomes and induction of LRRK2 kinase activity in response to lysosomal stress (By similarity).

#### **Cellular Location**

Cytoplasm. Preautophagosomal structure membrane; Peripheral membrane protein. Note=Colocalizes with nonmuscle actin. The conjugate detaches from the membrane immediately before or after autophagosome formation is completed (By similarity). Also localizes to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme. Under starved conditions, the ATG12-ATG5-ATG16L1 complex is translocated to phagophores driven by RAB33B (PubMed:32960676). {ECO:0000250, ECO:0000269|PubMed:32960676}

#### **Tissue Location**

Ubiquitous. The mRNA is present at similar levels in viable and apoptotic cells, whereas the protein is dramatically highly expressed in apoptotic cells

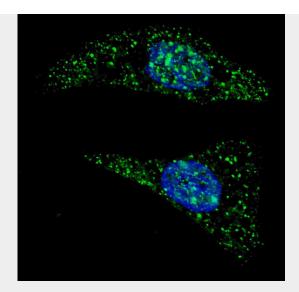
#### ATG5 Antibody (N-term) - Protocols

Provided below are standard protocols that you may find useful for product applications.

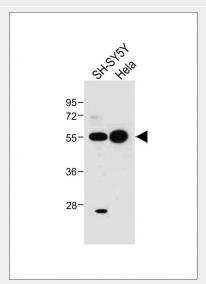
- Western Blot
- Blocking Peptides
- Dot Blot
- <u>Immunohistochemistry</u>
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

# ATG5 Antibody (N-term) - Images



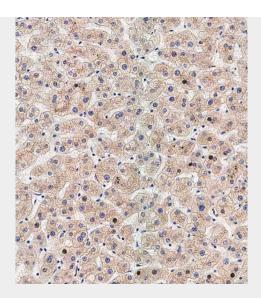


Fluorescent image of U251 cells stained with ATG5 (N-term) antibody. U251 cells were treated with Chloroquine (50  $\mu$ M,16h), then fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP1812a ATG5 (N-term) primary antibody (1:200, 2 h at room temperature). 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  $\mu$ g/ml, 5 min). ATG5 immunoreactivity is localized to autophagic vacuoles in the cytoplasm of U251 cells.

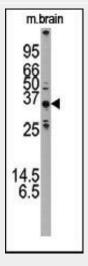


All lanes: Anti-hAPG5L-D3 at 1:1000 dilution Lane 1: SH-SY5Y whole cell lysate Lane 2: Hela whole cell lysate Lysates/proteins at 20  $\mu$ g per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size: 32 kDa Blocking/Dilution buffer: 5% NFDM/TBST.





Immunohistochemical analysis of paraffin-embedded human liver tissue using AP1812a performed on the Leica® BOND RXm. Tissue was fixed with formaldehyde at room temperature, antigen retrieval was by heat mediation with a EDTA buffer (pH9. 0). Samples were incubated with primary antibody(1:500) for 1 hours at room temperature. A undiluted biotinylated CRF Anti-Polyvalent HRP Polymer antibody was used as the secondary antibody.



The anti-APG5L Pab (Cat. #AP1812a) is used in Western blot to detect APG5L in mouse brain tissue lysate.

### ATG5 Antibody (N-term) - 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). APG5, required for autophagy, conjugates to ATG12 and associates with an isolation membrane to form a cup-shaped isolation membrane and autophagosome. The conjugate detaches from the membrane immediately before or after autophagosome formation is completed. APG5 may also play an important role in the apoptotic process, possibly within the modified cytoskeleton. Its expression is a relatively late event in the apoptotic process, occurring downstream of caspase activity.



## ATG5 Antibody (N-term) - 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)
- 6.Hammond E.M., et al. FEBS Lett. 425:391-395(1998)
- 7. Strausberg R.L., et al. PNAS 99:16899-16903(2002)
- 8.Grand R.J.A., et al. Exp. Cell Res. 218:439-451(1995)
- 9. Mizushima N., et al. J. Biol. Chem. 273:33889-33892(1998)
- 10. Mizushima N., et al. J. Cell Biol. 152:657-668(2001)

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].

### **ATG5 Antibody (N-term) - Citations**

- Pitavastatin activates mitophagy to protect EPC proliferation through a calcium-dependent CAMK1-PINK1 pathway in atherosclerotic mice
- The Dynein Adaptor RILP Controls Neuronal Autophagosome Biogenesis, Transport, and Clearance
- <u>Isolation of Rab5-positive endosomes reveals a new mitochondrial degradation pathway</u> utilized by BNIP3 and Parkin
- ATG5 Promotes Death Signaling in Response to the Cyclic Depsipeptides Coibamide A and Apratoxin A.
- Atg5flox-Derived Autophagy-Deficient Model of Pompe Disease: Does It Tell the Whole Story?
- The Detection Techniques for Autophagy-Associated Cell Death-Related Genes and Proteins: Gene Expression Assay and Immunohistochemistry.
- Effects of Combined Lysosomal and Mitochondrial Photodamage in a Non Small-Cell Lung Cancer Cell Line: the Role of Paraptosis.
- Studying Autophagy in Zebrafish.
- <u>Promotion of Pro-Apoptotic Signals by Lysosomal Photodamage: Mechanistic Aspects and Influence of Autophagy.</u>
- FGFR3/Fibroblast Growth Factor Receptor 3 Inhibits Autophagy through Decreasing the ATG12-ATG5 Conjugate, Leading to the Delay of Cartilage Development in Achondroplasia.
- Autophagy in spinal motor neurons of conditional ADAR2-knockout mice: an implication for a role of calcium in increased autophagy flux in ALS.
- Long-term artificial selection reveals a role of TCTP in autophagy in mammalian cells.
- Coxsackievirus B3 induces crosstalk between autophagy and apoptosis to benefit its release
  after replicating in autophagosomes through a mechanism involving caspase cleavage of
  autophagy-related proteins.
- Control of photoreceptor autophagy after retinal detachment: the switch from survival to death.
- Autophagosomes contribute to intracellular lipid distribution in enterocytes.
- Autophagy Induced by Tumor Necrosis Factor α Mediates Intrinsic Apoptosis in Trophoblastic Cells.
- Down-regulation of autophagy-related protein 5 (ATG5) contributes to the pathogenesis of





early-stage cutaneous melanoma.

- Phosphorylation of Atg5 by the Gadd45Î<sup>2</sup>-MEKK4-p38 pathway inhibits autophagy.
- Cell loss and autophagy in the extra-adrenal chromaffin organ of Zuckerkandl are regulated by glucocorticoid signalling.
- Arsenic trioxide enhances the radiation sensitivity of androgen-dependent and -independent human prostate cancer cells.
- Autophagy induced by deficiency of sphingosine-1-phosphate phosphohydrolase 1 is switched to apoptosis by calpain-mediated autophagy-related gene 5 (Atg5) cleavage.
- Expression pattern and functions of autophagy-related gene atg5 in zebrafish organogenesis.
- Arsenic trioxide induces autophagy and apoptosis in human glioma cells in vitro and in vivo through downregulation of survivin.
- A rapid method to improve protein detection by indirect ELISA.
- p62, Ref(2)P and ubiquitinated proteins are conserved markers of neuronal aging, aggregate formation and progressive autophagic defects.
- Sphingosine-1-phosphate phosphohydrolase-1 regulates ER stress-induced autophagy.
- Control of basal autophagy by calpain1 mediated cleavage of ATG5.
- An increase in intracellular Ca2+ is required for the activation of mitochondrial calpain to release AIF during cell death.