

**ATG4D Antibody (C-term)**  
**Purified Rabbit Polyclonal Antibody (Pab)**  
**Catalog # AP1811c****Specification**

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**ATG4D Antibody (C-term) - Product Information**

Application	IF, WB, IHC-P,E
Primary Accession	<a href="#">Q86TL0</a>
Reactivity	Human
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Antigen Region	441-470

**ATG4D Antibody (C-term) - Additional Information****Gene ID** 84971**Other Names**

Cysteine protease ATG4D, 3422-, AUT-like 4 cysteine endopeptidase, Autophagin-4, Autophagy-related cysteine endopeptidase 4, Autophagy-related protein 4 homolog D, Cysteine protease ATG4D, mitochondrial, ATG4D, APG4D, AUTL4

**Target/Specificity**

This ATG4D antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 441-470 amino acids from the C-terminal region of human ATG4D.

**Dilution**

IF~~1:100  
WB~~1:1000  
IHC-P~~1:50~100

**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**

ATG4D Antibody (C-term) is for research use only and not for use in diagnostic or therapeutic procedures.

**ATG4D Antibody (C-term) - Protein Information****Name** ATG4D {ECO:0000303|PubMed:19549685, ECO:0000312|HGNC:HGNC:20789}

**Function** [Cysteine protease ATG4D]: Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins (PubMed:[21177865](#), PubMed:[29458288](#), PubMed:[30661429](#)). The protease activity is required for proteolytic activation of ATG8 family proteins: cleaves the C-terminal amino acid of ATG8 proteins MAP1LC3 and GABARAPL2, to reveal a C-terminal glycine (PubMed:[21177865](#)). 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 (By similarity). In addition to the protease activity, also mediates delipidation of ATG8 family proteins (PubMed:[29458288](#), PubMed:[33909989](#)). Catalyzes delipidation of PE-conjugated forms of ATG8 proteins during macroautophagy (PubMed:[29458288](#), 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](#)). ATG4D plays a role in the autophagy-mediated neuronal homeostasis in the central nervous system (By similarity). Compared to other members of the family (ATG4A, ATG4B or ATG4C), constitutes the major protein for the delipidation activity, while it promotes weak proteolytic activation of ATG8 proteins (By similarity). 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**

[Cysteine protease ATG4D]: Cytoplasm

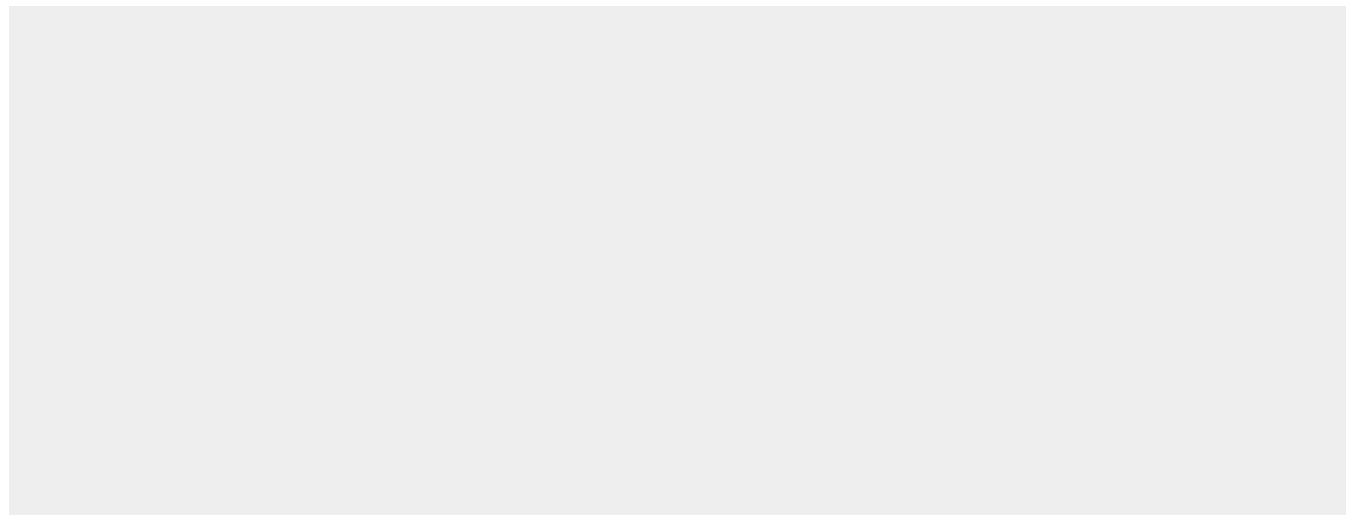
**Tissue Location**

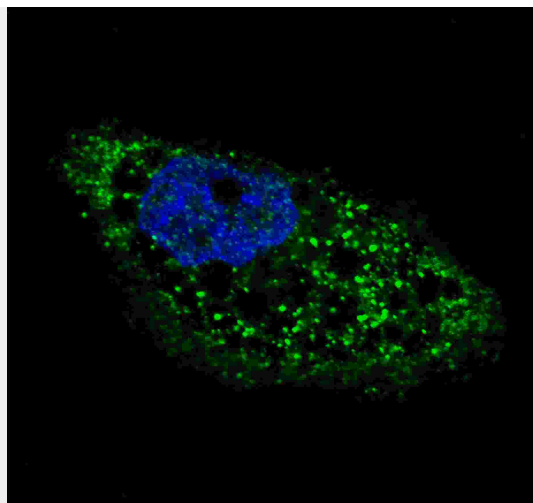
Widely expressed in testis.

**ATG4D Antibody (C-term) - Protocols**

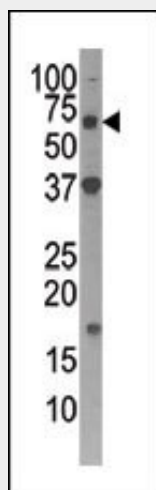
Provided below are standard protocols that you may find useful for product applications.

- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

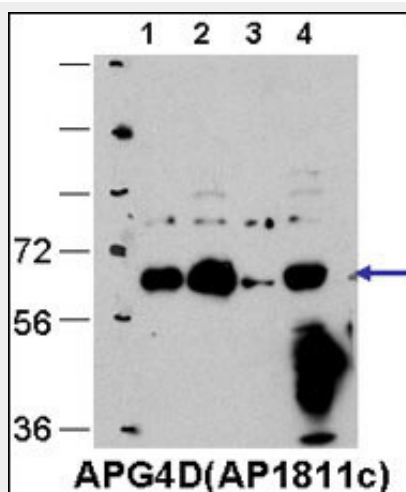
**ATG4D Antibody (C-term) - Images**



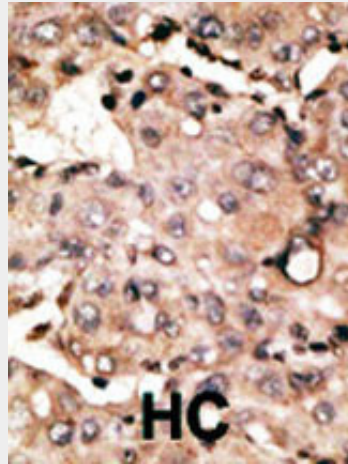
Fluorescent image of U251 cells stained with ATG4D (C-term) antibody. U251 cells were treated with Chloroquine (50  $\mu$ M, 16h), then fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP1811c ATG4D (C-term) primary antibody (1:100, 2 h at room 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  $\mu$ g/ml, 5 min). ATG4D immunoreactivity is localized to autophagic vacuoles in the cytoplasm of U251 cells.



Western blot analysis of APG4D Pab in Y79 cell lysate (Cat. #AP1811c).



Cos7, HEK293, MEF, and Hela cells, left to right respectively. Data courtesy of Drs. Jiefei Geng and Dan Klionsky, University of Michigan.



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.

#### **ATG4D Antibody (C-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).

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.

#### **ATG4D Antibody (C-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)

References for U251 cell line:

1. Westermarck 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., Westermarck 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].

