10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

ATG4A Antibody

Purified Mouse Monoclonal Antibody (Mab)
Catalog # AM8455b

Product Information

Application	WB, IHC-P, IF, FC, E
Primary Accession	Q8WYN0
Reactivity	Human
Host	Mouse
Clonality	monoclonal
Isotype	IgG2b,k
Clone Names	1458CT808.66.25.69
Calculated MW	45378

Additional Information

Gene ID	115201
Other Names	Cysteine protease ATG4A, 3422-, AUT-like 2 cysteine endopeptidase, Autophagin-2, Autophagy-related cysteine endopeptidase 2, Autophagy-related protein 4 homolog A, hAPG4A, ATG4A, APG4A, AUTL2
Target/Specificity	This ATG4A antibody is generated from a mouse immunized with a recombinant protein.
Dilution	WB~~1:500 IHC-P~~1:100~500 IF~~1:25 FC~~1:25 E~~Use at an assay dependent concentration.
Format	Purified monoclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein G column, 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	ATG4A Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	ATG4A {ECO:0000303|Ref.20, ECO:0000312|HGNC:HGNC:16489}
Function	Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004,

PubMed:32732290). The protease activity is required for proteolytic activation of ATG8 family proteins: cleaves the C-terminal amino acid of ATG8 proteins to reveal a C-terminal glycine (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004). 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:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004). Preferred substrate is GABARAPL2 followed by MAP1LC3A and GABARAP (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, 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:29458288, PubMed:33909989). Catalyzes delipidation of PE-conjugated forms of ATG8 proteins during macroautophagy (PubMed:29458288, PubMed:33909989). Compared to ATG4B, the major protein for proteolytic activation of ATG8 proteins, shows weaker ability to cleave the C-terminal amino acid of ATG8 proteins, while it displays stronger delipidation activity (PubMed:29458288). 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 {ECO:0000250|UniProtKB:Q8BGE6}.

Background

Cysteine protease required for the cytoplasm to vacuole transport (Cvt) and autophagy. Cleaves the C-terminal amino acid of ATG8 family proteins to reveal a C-terminal glycine. 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. Preferred substrate is GABARAPL2 followed by MAP1LC3A and GABARAP. Has also an activity of delipidating enzyme for the PE-conjugated forms.

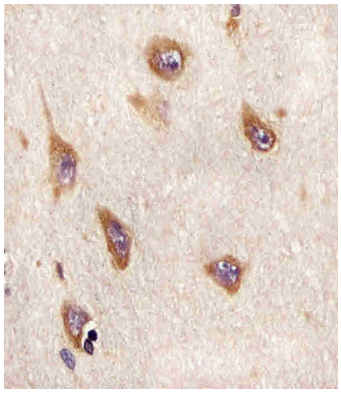
References

Marino G.,et al.J. Biol. Chem. 278:3671-3678(2003).
Kabeya Y.,et al.J. Cell Sci. 117:2805-2812(2004).
Chen J.M.,et al.Submitted (SEP-2001) to the EMBL/GenBank/DDBJ databases.
Ota T.,et al.Nat. Genet. 36:40-45(2004).
Ross M.T.,et al.Nature 434:325-337(2005).

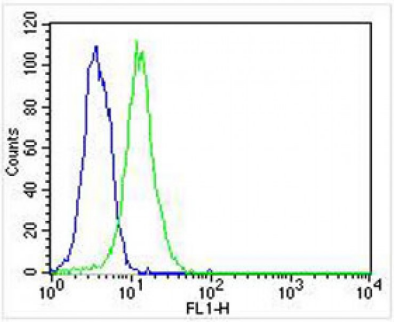
Images

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical epithelial adenocarcinoma cell line) cells labeling ATG4A with AM8455b at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-mouse IgG (NA166821) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm staining on HeLa cell line. Cytoplasmic actin is detected

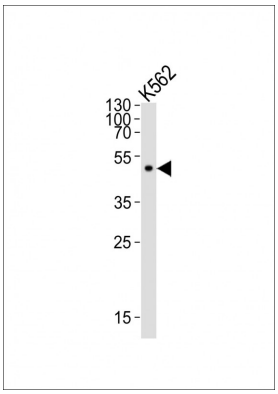
with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red).The nuclear counter stain is DAPI (blue).



AM8455b staining ATG4A in human brain sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.



Overlay histogram showing Hela cells stained with AM8455b (green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AM8455b, 1:25 dilution) for 60 min at 37ºC. The secondary antibody used was Goat-Anti-Mouse IgG, DyLight® 488 Conjugated Highly Cross-Adsorbed(NA168821)) at 1/400 dilution for 40 min at 37ºC. Isotype control antibody (blue line) was mouse IgG2b (1µg/1x10^6 cells) used under the same conditions. Acquisition of >10, 000 events was performed.



Western blot analysis of lysate from K562 cell line, using ATG4A Antibody(Cat. #AM8455b). AM8455b was diluted at 1:500. A goat anti-mouse IgG H&L(HRP) at 1:10000 dilution was used as the secondary antibody. Lysate at 20µg.