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Datasheet for 200-401-H57

ATG8 Antibody

Overview

Description:	Anti-ATG8 (RABBIT) Antibody - 200-401-H57
Item No.:	200-401-H57
Size:	100 µg
Applications:	IHC, WB, IF
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

Product Details

Background:	Anti-ATG8 antibody detects human ATG8. MAP1A and MAP1B are microtubule-associated proteins made up of a heavy chain and multiple light chain components. One such light chain component associated with these proteins is ATG8. ATG8 (also called MAP1LC3A and APG8), an ~16KDa mammalian homolog of yeast Atg8, is a cytoplasmic protein that plays an essential role in the process and regulation of autophagy. ATG8 is an evolutionarily conserved protein that belongs to LC3 family. ATG8 interacts with various proteins in the cytoplasm including mammalian homologues of Atg4, Atg7, Atg3 and Atg12 and gets modified into LC3-I and LC3-II respectively. Both of these forms are suggested to be involved in the formation and intracellular trafficking of autophagosomes in mammalian cells. Anti-ATG8 antibody are ideal for investigators involved in apoptosis research.
Synonyms:	Activating transcription factor 8
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	MAP1LC3A
Reactivity:	Human, Mouse, Rat
Immunogen Type:	Conjugated Peptide

Immunogen:	ATG8 Antibody was produced from whole rabbit serum prepared by repeated immunizations with at the n-terminus portion of human ATG8.
Purity/Specificity:	Anti-ATG8 Antibody was purified by Protein G chromatography. A BLAST analysis was used to suggest cross-reactivity with Anti-ATG8 from Opossum and rat based on 100% homology with the immunizing sequence. Cross-reactivity with Anti-ATG8 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q9H492 • NCBI - NP_115903.1 • GeneID - 84557

Application Details

Tested Applications:	IHC, WB
Suggested Applications:	IF (Based on references)
Application Note:	Anti-ATG8 antibody is tested by IHC-P and WB. Expect a band approximately 16 kDa on specific lysates. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IHC:	5-10 µg/mL
WB:	0.25-1 µg/mL

Formulation

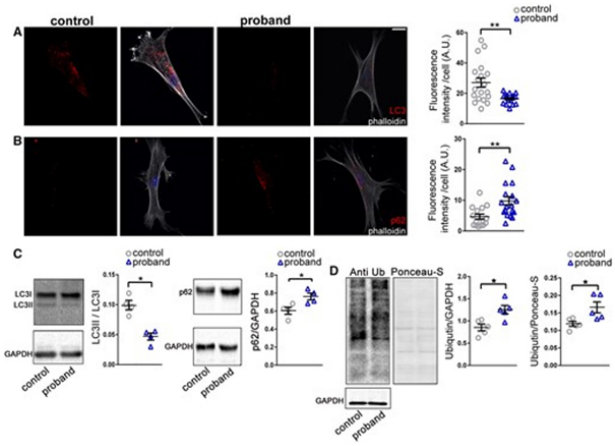
Physical State:	Liquid
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.05% (w/v) Sodium Azide

Shipping & Handling

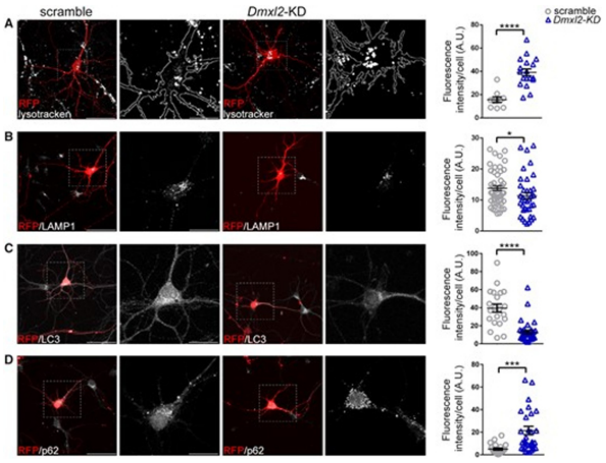
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

Expiration: Expiration date is one (1) year from date of receipt.

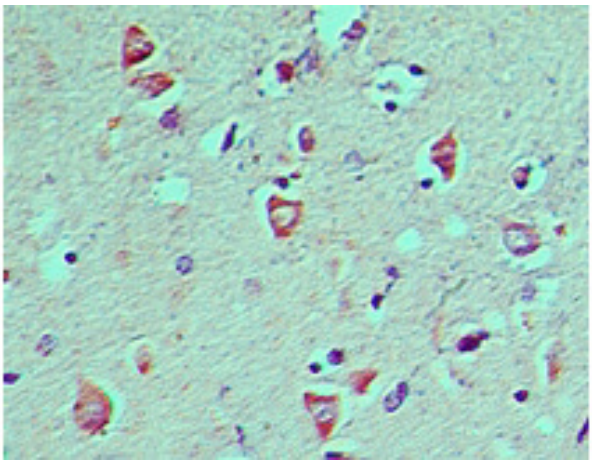
Images



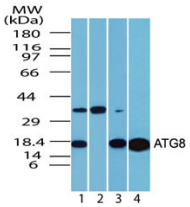
Immunofluorescence Microscopy
Autophagy defects in Patient 1-II:1's cells.
(A) Left: Representative images of fibroblasts from Patient 1-II:1 (proband) and the respective control immunolabelled with LC3 and stained with phalloidin. Scale bar = 20 µm. Right: LC3 fluorescence intensity (individual data and means ± SEM) was quantified in 19 (control) and 16 (proband) cells. **P < 0.01; unpaired t-test with Welch's correction. (B) Left: Representative images of fibroblasts as in A, immunolabelled with p62 and stained with phalloidin. Right: p62 fluorescence intensity (individual data and means ± SEM) was quantified in 15 (control) and 18 (proband) cells. **P < 0.005; unpaired t-test with Welch's correction. (C) Representative western blot of LC3I, LC3II, p62 and GAPDH obtained from fibroblast lysates (30 µg). Immunoreactivity was quantified by densitometric analysis and normalized to LC3I for LC3II, and to GAPDH for p62. Data are means ± SEM from n = 4 independent experiments. *P < 0.05; Mann-Whitney U-test. (D) Representative western blot from fibroblast lysates (30 µg). Ubiquitinated proteins were detected with anti-ubiquitin (Anti-Ub) antibody; GAPDH or Ponceau-S intensity along the lanes were used as loading controls. Ubiquitinated proteins immunoreactivity was quantified by densitometric analysis and normalized to GAPDH or to Ponceau S. Data are means ± SEM from n = 5 independent experiments. *P < 0.05; Mann-Whitney U-test. A.U. = arbitrary units of fluorescence intensity. Fig 4.
PMID: 31688942



Immunofluorescence Microscopy
Loss of neuronal Dmxl2 alters lysosomal and autophagy markers. (A) Left: Representative images of hippocampal mouse neurons transfected at 4 DIV with control shRNA (scramble) or Dmxl2 shRNA (Dmxl2-KD) harbouring the red fluorescent protein (RFP) reporter and incubated with LysoTracker® (50 nM, 30 min) at 7 DIV. Manual tracing of neuronal surface is shown. Scale bar = 50 µm. White dashed rectangles indicate regions shown at high magnification on the right. Scale bar = 125 µm. Right: LysoTracker® fluorescence intensity (individual data and means ± SEM) was quantified in 10 (scramble) and 18 (Dmxl2-KD) cells from three independent preparations. (B–D) Left: Representative images of hippocampal mouse neurons as in A and immunostained for LAMP1 (B), LC3 (C) or p62 (D). Scale bar = 50 µm. White dashed rectangles indicate regions shown at high magnification on the right. Scale bar = 100 µm. Right: Respective fluorescence intensity (individual data and means ± SEM) was quantified in 21–37 (scramble) and 27–33 (Dmxl2-KD) cells from three independent preparations. A.U. = arbitrary units of fluorescence intensity. *P < 0.05, ***P < 0.001, ****P < 0.0001 Mann-Whitney unpaired t-test. Fig 5. PMID: 31688942



Immunohistochemistry
Immunohistochemistry of Rabbit Anti-ATG8 antibody. Tissue: human brain. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: ATG8 antibody at 5 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT.
Staining: ATG8 is stained brown.



Western Blot
Western Blot of Rabbit Anti-ATG8 antibody. Lane 1: Lysate from human brain without an immunizing peptide. Lane 2: human brain with immunizing peptide. Lane 3: mouse brain with antibody. Lane 4: rat brain with antibody. Primary antibody: ATG8 at 1 µg/mL overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 80 kDa for ATG8. Other band(s): none.

References

- Esposito A et al. Biallelic DMXL2 mutations impair autophagy and cause Ohtahara syndrome with progressive course. *Brain.* (2019)

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