Anti-ATG4A Antibody [JE32-31]

HA721010

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Cell
Molecular Wt:	Predicted band size: 45 kDa
Clone number:	JE32-31

Description: Cysteine protease ATG4A is an enzyme that in humans is encoded by the ATG4A gene. Autophagy is the process by which endogenous proteins and damaged organelles are destroyed intracellularly. Autophagy is postulated to be essential for cell homeostasis and cell remodelling during differentiation, metamorphosis, non-apoptotic cell death, and aging. Reduced levels of autophagy have been described in some malignant tumors, and a role for autophagy in controlling the unregulated cell growth linked to cancer has been proposed. This gene encodes a member of the autophagin protein family. The encoded protein is also designated as a member of the C-54 family of cysteine proteases. Transcript variants that encode distinct isoforms have been identified.

Immunogen: Synthetic peptide within human ATG4A aa 1-50/398.

Positive control: K562 cell lysate, Jurkat cell lysate, mouse kidney tissue lysate, rat kidney tissue lysate, human testis tissue, human striated muscle tissue, mouse kidney tissue, PC-3M, Hela.

Subcellular location: Cytoplasm.

Database links: SwissProt: Q8WYN0 Human | Q8C9S8 Mouse | B1H274 Rat

Recommended Dilutions:

WB	1:500
IHC-P	1:100-1:400
FC	1:500-1:1,000
IF-Cell	1:200

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

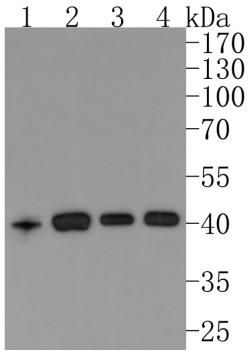

Fig1: Western blot analysis of ATG4A on different lysates with Rabbit anti-ATG4A antibody (HA721010) at 1/500 dilution.

Lane 1: K562 cell lysate, 10 µg/Lane
Lane 2: Jurkat cell lysate, 10 µg/Lane
Lane 3: Mouse kidney tissue lysate, 20 µg/Lane
Lane 4: Rat kidney tissue lysate, 20 µg/Lane

Predicted band size: 45 kDa
Observed band size: 41 kDa

Exposure time: 2 minutes;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721010) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

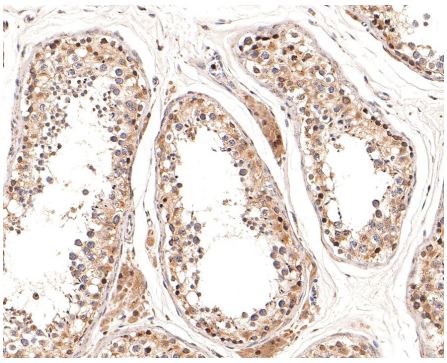

Fig2: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-ATG4A antibody (HA721010) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721010) at 1/100 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

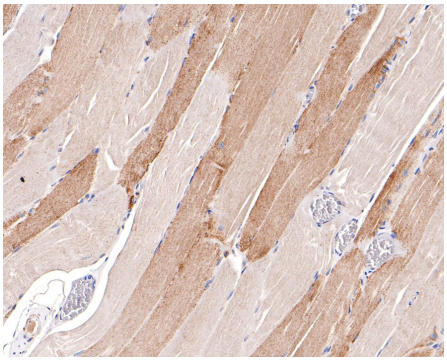

Fig3: Immunohistochemical analysis of paraffin-embedded human striated muscle tissue with Rabbit anti-ATG4A antibody (HA721010) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721010) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

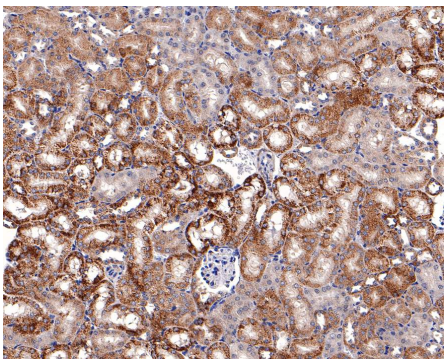

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-ATG4A antibody (HA721010) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721010) at 1/100 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

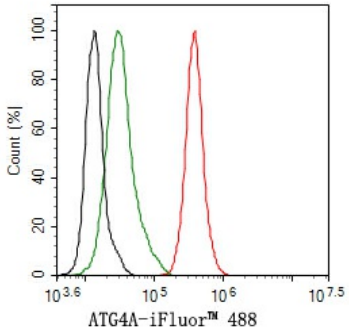

Fig5: Flow cytometric analysis of PC-3M cells labeling ATG4A.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721010, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

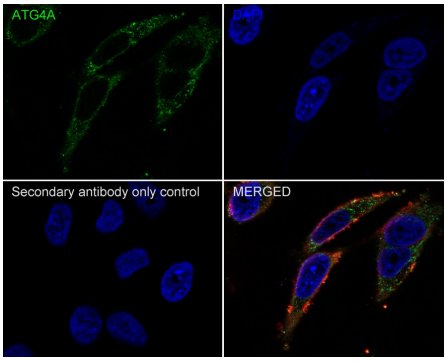

Fig6: Immunocytochemistry analysis of Hela cells labeling ATG4A with Rabbit anti-ATG4A antibody (HA721010) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-ATG4A antibody (HA721010) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Hait AS. et. al. Defects in LC3B2 and ATG4A underlie HSV2 meningitis and reveal a critical role for autophagy in antiviral defense in humans. Sci Immunol. 2020 Dec
2. Nguyen N. et. al. The insufficiency of ATG4A in macroautophagy. J Biol Chem. 2020 Sep