Anti-ATG9A Antibody [SC67-05]

ET1610-71

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IP
Molecular Wt:	Predicted band size: 94 kDa
Clone number:	SC67-05

Description: Autophagy, a process that results in the lysosomal-dependent degradation of cytosolic compartments, is carried out by the autophagosome, which is a double-membrane vesicle whose formation is catalyzed by several autophagy-related gene (Atg) proteins. Atg9a (autophagy-related protein 9A), also known as APG9-like 1, is a 839 amino acid, multi-pass membrane protein that localizes to the pre-autophagosomal structure (PAS). Isolation membranes are suggested to originate from the PAS, enwrapping cytoplasmic components to form a double membrane autophagosome, which then fuses with the vacuole. Ubiquitously expressed in human adult tissues, Atg9a cycles between the Golgi and endosomes and, with the autophagosome-specific marker LC3, plays a critical role in starvation-induced autophagosome formation. Three isoforms of Atg9a exist as a result of alternative splicing events.

Immunogen: Synthetic peptide within Human ATG9A aa 799-839 / 839.

Positive control: NCI-H1299 cell lysate, Neuro-2a cell lysate, Human brain tissue lysate, Mouse brain tissue lysate, Rat brain tissue lysate, mouse brain tissue, rat brain tissue.

Subcellular location: Cytoplasmic vesicle, Golgi apparatus, Late endosome membrane, Endoplasmic reticulum membrane.

Database links: SwissProt: Q7Z3C6 Human | Q68FE2 Mouse | Q5FWU3 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

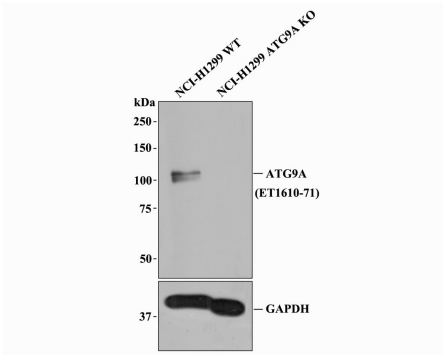

Fig1: All lanes: Western blot analysis of ATG9A with anti-ATG9A antibody [SC67-05] (ET1610-71) at 1:1,000 dilution.
Lane 1: Wild-type NCI-H1299 whole cell lysate (20 µg).
Lane 2: ATG9A knockout NCI-H1299 whole cell lysate (20 µg).

ET1610-71 was shown to specifically react with ATG9A in wild-type NCI-H1299 cells. No band was observed when ATG9A knockout sample was tested. Wild-type and ATG9A knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1610-71, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) was used in 5% BSA 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.

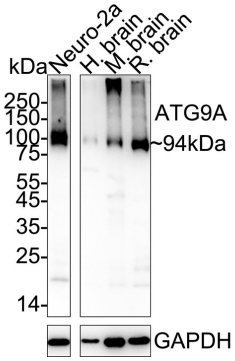

Fig2: Western blot analysis of ATG9A on different lysates with Rabbit anti-ATG9A antibody (ET1610-71) at 1/1,000 dilution.

Lane 1: Neuro-2a cell lysate
Lane 2: Human brain tissue lysate
Lane 3: Mouse brain tissue lysate
Lane 4: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 94 kDa
Observed band size: 94 kDa

Exposure time: 20 seconds; ECL: K1802;

4-20% 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 (ET1610-71) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

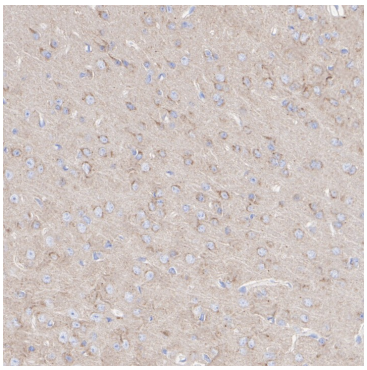

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-ATG9A antibody (ET1610-71) at 1/200 dilution.

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 (ET1610-71) at 1/200 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.

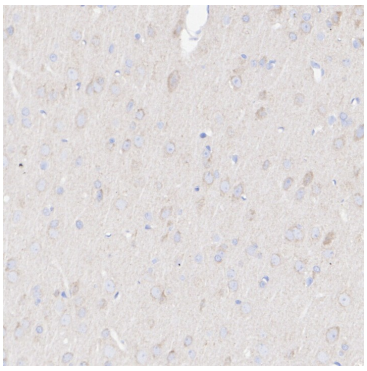

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-ATG9A antibody (ET1610-71) at 1/200 dilution.

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 (ET1610-71) at 1/200 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.

Background References

1. Moreau K et al. Methods to analyze SNARE-dependent vesicular fusion events that regulate autophagosome biogenesis. Methods 75:19-24 (2015).
2. Zavodszky E et al. Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. Nat Commun 5:3828 (2014).