

Product Datasheet

ATG14L Rabbit Polyclonal Antibody (orb308776)

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| Catalog Number | orb308776 |
| Category | Antibodies |
| Description | ATG14L Rabbit Polyclonal Antibody |
| Target | Beclin 1-associated autophagy-related key regulator |
| Clonality | Polyclonal |
| Species/Host | Rabbit |
| Isotype | Rabbit IgG |
| Conjugation | Unconjugated |
| Reactivity | Human, Rat |
| Form/Appearance | Lyophilized |
| Concentration | Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml. |
| Buffer/Preservatives | Each vial contains antibody formulated with stabilizing components, 0.9 mg NaCl, 0.2 mg Na ₂ HPO ₄ , and 0.05 mg NaN ₃ . *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required. |
| Reconstitution | Add 0.2ml of distilled water will yield a concentration of 500ug/ml. |
| Purification | Immunogen affinity purified. |

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|----------------------------|---|
| Immunogen | A synthetic peptide corresponding to a sequence at the N-terminus of human ATG14L, different from the related mouse and rat sequences by two amino acids. |
| UniProt ID | Q6ZNE5 |
| MW | 59 kDa |
| Tested applications | FC, ICC, IF, IHC, WB |
| Dilution range | Western blot, 0.1-0.5µg/ml, Human, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml, Human, Rat Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Immunofluorescence, 5 µg/ml, Human, Rat Flow Cytometry (Fixed), 1-3µg/1x10 ⁶ cells, Human |
| Specificity | No cross reactivity with other proteins. |
| Cross Reactivity | No cross-reactivity with other proteins |
| Antibody Type | Primary Antibody |
| 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. |
| Note | For research use only |
| Expiration Date | 12 months from date of receipt. |

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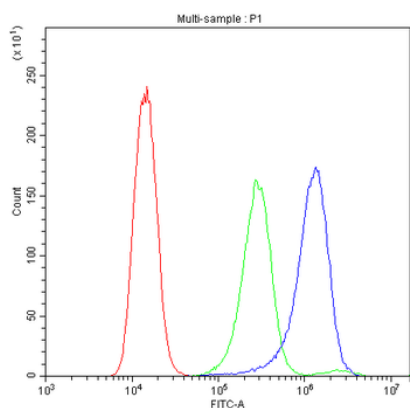
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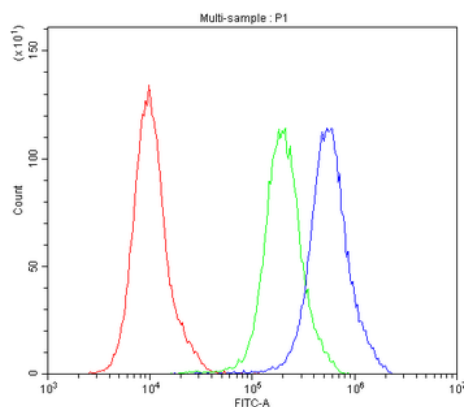
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Flow Cytometry analysis of A431 cells using anti-ATG14L antibody. Overlay histogram showing A431 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ATG14L Antibody (1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of PC-3 cells using anti-ATG14L antibody. Overlay histogram showing PC-3 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ATG14L Antibody (1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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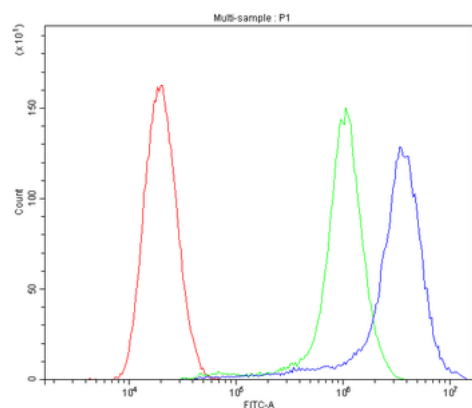
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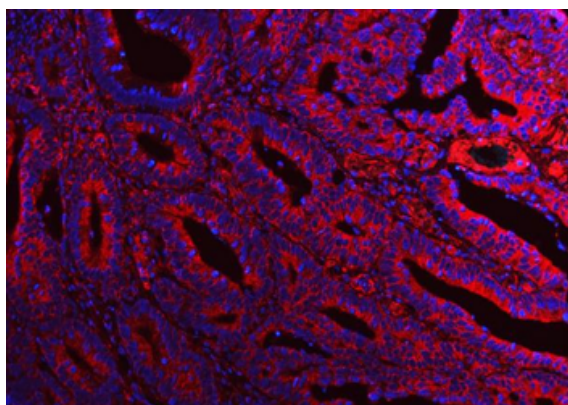
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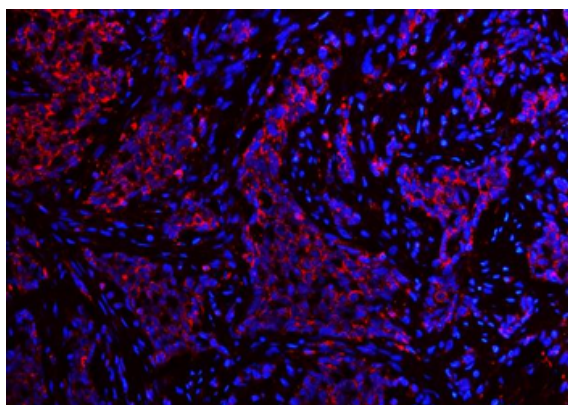
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Flow Cytometry analysis of SiHa cells using anti-ATG14L antibody. Overlay histogram showing SiHa cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ATG14L Antibody (1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of ATG14L using anti-ATG14L antibody. ATG14L was detected in a paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 $\mu\text{g}/\text{mL}$ rabbit anti-ATG14L Antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of ATG14L using anti-ATG14L antibody. ATG14L was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 $\mu\text{g}/\text{mL}$ rabbit anti-ATG14L Antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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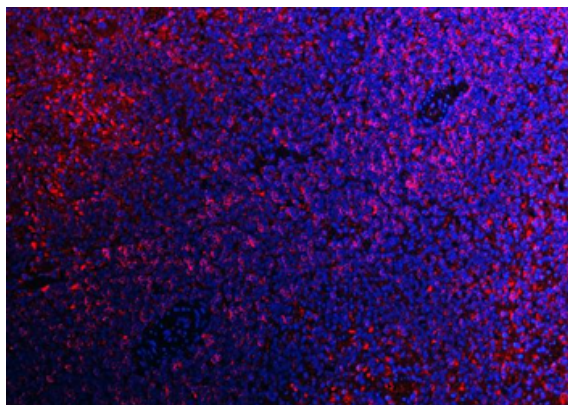
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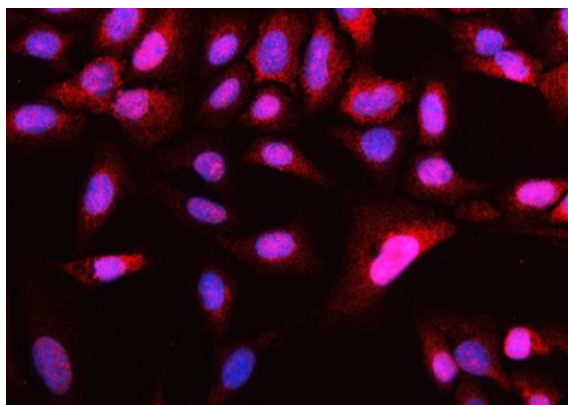
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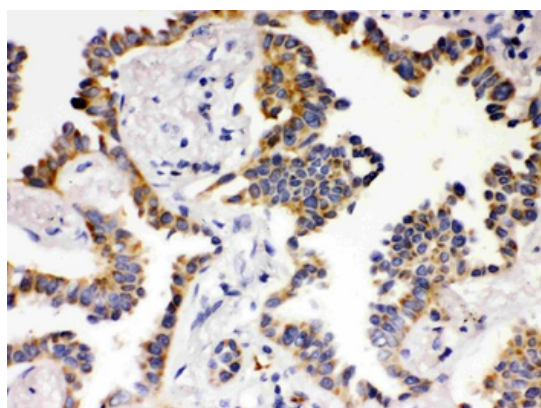
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IF analysis of ATG14L using anti-ATG14L antibody. ATG14L was detected in a paraffin-embedded section of rat spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-ATG14L Antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of ATG14L using anti-ATG14L antibody. ATG14L was detected in an immunocytochemical section of U2OS cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL rabbit anti-ATG14L Antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IHC analysis of ATG14L using anti-ATG14L antibody. ATG14L was detected in paraffin-embedded section of Human Lung Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml rabbit anti-ATG14L Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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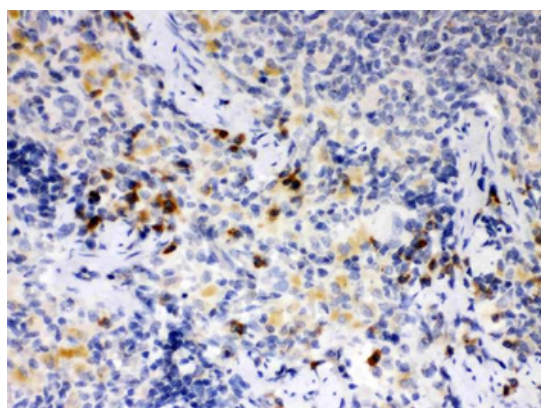
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IHC analysis of ATG14L using anti-ATG14L antibody. ATG14L was detected in paraffin-embedded section of Rat Spleen Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-ATG14L Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



Western blot analysis of ATG14L using anti-ATG14L antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 μ g of sample under reducing conditions. Lane 1: Rat Brain Tissue Lysate, Lane 2: HELA Whole Cell Lysate. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ATG14L antigen affinity purified polyclonal antibody at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for ATG14L at approximately 59 KD. The expected band size for ATG14L is at 59 KD.

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