

## Product Datasheet

### Anti-Beclin 1 Antibody (monoclonal, 2D12A3) (orb1145791)

<b>Description</b>	Anti-Beclin 1 Antibody (monoclonal, 2D12A3). Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Mouse
<b>Reactivity</b>	Human, Mouse, Rat
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	FC, ICC, IF, IHC, WB
<b>Immunogen</b>	E.coli-derived human Beclin 1 recombinant protein (Position: M1-S354). Human Beclin 1 shares 97% amino acid (aa) sequence identity with both mouse and rat Beclin 1.
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Storage</b>	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.
<b>Note</b>	For research use only
<b>Application notes</b>	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x10 <sup>6</sup> cells, Human. Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml
<b>Isotype</b>	Mouse IgG1
<b>Clonality</b>	Monoclonal
<b>Clone Number</b>	2D12A3

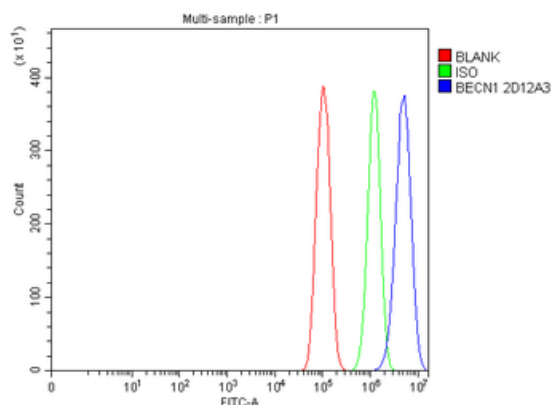
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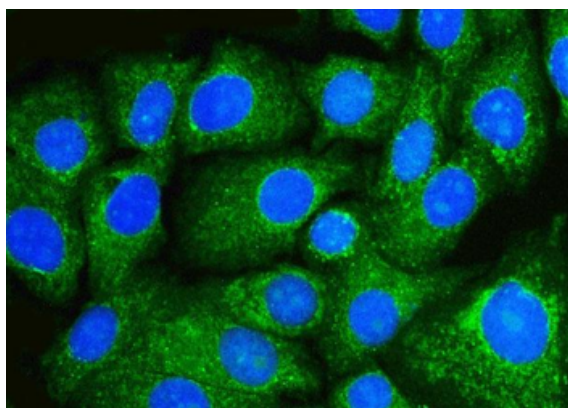
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<b>Antibody Type</b>	Primary Antibody
<b>MW</b>	52-60 kDa
<b>Uniprot ID</b>	<b>Q14457</b>
<b>Expiration Date</b>	12 months from date of receipt.



Flow Cytometry analysis of PC-3 cells using anti-Beclin 1 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 mouse anti-Beclin 1 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse 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 mouse 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.



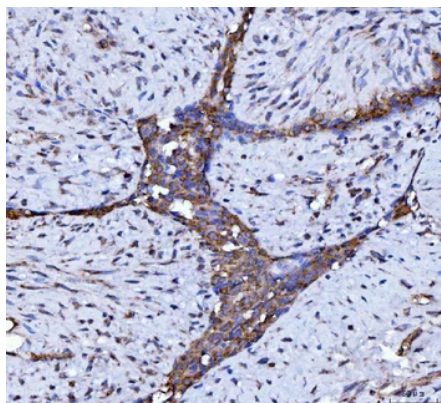
IF analysis of Beclin 1 using anti-Beclin 1 antibody. Beclin 1 was detected in an immunocytochemical section of HepG2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5  $\mu\text{g}/\text{mL}$  mouse anti-Beclin 1 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG was used as secondary antibody at 1:100 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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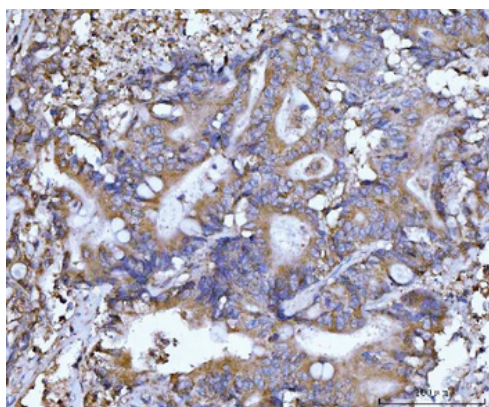
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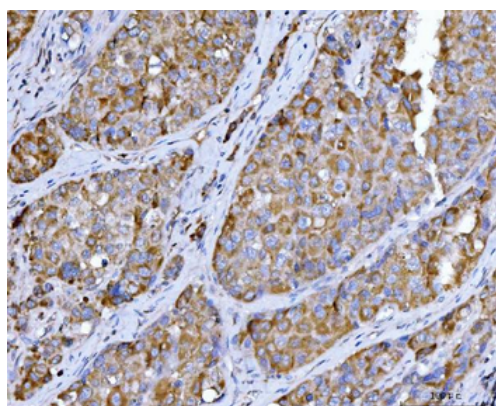
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IHC analysis of Beclin 1 using anti-Beclin 1 antibody. Beclin 1 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Beclin 1 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of Beclin 1 using anti-Beclin 1 antibody. Beclin 1 was detected in a paraffin-embedded section of human colonic adenoma 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 2 µg/ml mouse anti-Beclin 1 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit with DAB as the chromogen.



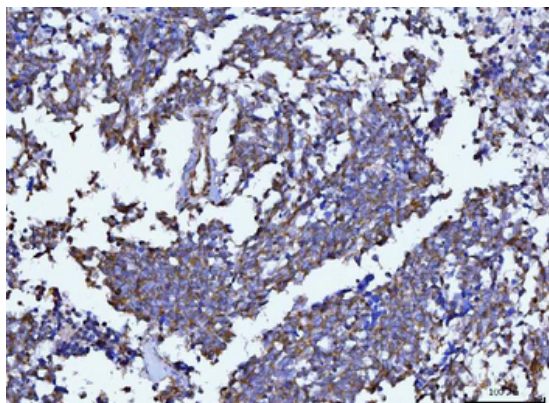
IHC analysis of Beclin 1 using anti-Beclin 1 antibody. Beclin 1 was detected in a paraffin-embedded section of human hepatocellular carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Beclin 1 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit with DAB as the chromogen.

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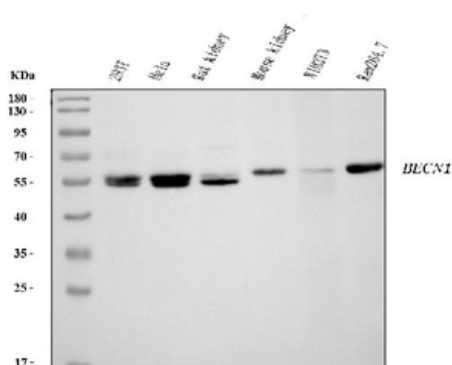
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IHC analysis of Beclin 1 using anti-Beclin 1 antibody. Beclin 1 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu$ g/ml mouse anti-Beclin 1 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit with DAB as the chromogen.



Western blot analysis of Beclin 1 using anti-Beclin 1 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 30  $\mu$ g of sample under reducing conditions. Lane 1: human 293T whole cell lysates, Lane 2: human HeLa whole cell lysates, Lane 3: rat kidney tissue lysates, Lane 4: mouse kidney tissue lysates, Lane 5: mouse NIH/3T3 whole cell lysates, Lane 6: mouse RAW264.7 whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Beclin 1 antigen affinity purified monoclonal antibody at 0.5  $\mu$ 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-mouse 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 Beclin 1 at approximately 52-60 kDa. The expected band size for Beclin 1 is at 52 kDa.

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