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Anti-Beclin 1 Antibody Picoband™ (monoclonal, 2D12A3)

Catalog # ABO16255

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q14457
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Beclin 1 Antibody Picoband™ (monoclonal, 2D12A3) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	8678
Other Names	Beclin-1, Coiled-coil myosin-like BCL2-interacting protein, Protein GT197, Beclin-1-C 35 kDa, Beclin-1-C 37 kDa, BECN1, GT197
Calculated MW	51896
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 2D12A3
Immunogen	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.
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

Protein Information

Name	BECN1
Synonyms	GT197
Function	Plays a central role in autophagy (PubMed: 18570871, PubMed:21358617, PubMed:23184933, PubMed:23974797, PubMed:25484083, PubMed:28445460, PubMed:37776275). Acts as a core subunit of the PI3K complex that mediates formation of phosphatidylinositol 3-phosphate; different complex forms are believed to play a role in multiple membrane trafficking pathways: PI3KC3-C1 is involved in initiation of autophagosomes and PI3KC3-C2 in maturation of autophagosomes and endocytosis. Involved in regulation of degradative endocytic trafficking and required for the abscission step in cytokinesis, probably in the context of PI3KC3-C2 (PubMed:20208530, PubMed:20643123, PubMed:23974797, PubMed:26783301). Essential for the formation of PI3KC3-C2 but not PI3KC3-C1 PI3K complex forms. Involved in endocytosis (PubMed:25275521). May play a role in antiviral host defense.
Cellular Location	Cytoplasm. Golgi apparatus, trans-Golgi network membrane; Peripheral membrane protein. Endosome membrane; Peripheral membrane protein. Endoplasmic reticulum membrane; Peripheral membrane protein. Mitochondrion membrane; Peripheral membrane protein. Endosome {ECO:0000250|UniProtKB:O88597} Cytoplasmic vesicle, autophagosome. Note=Interaction with ATG14 promotes translocation to autophagosomes. Expressed in dendrites and cell bodies of cerebellar Purkinje cells (By similarity) {ECO:0000250|UniProtKB:O88597, ECO:0000269|PubMed:19050071} [Beclin-1-C 37 kDa]: Mitochondrion {ECO:0000250|UniProtKB:O88597}
Tissue Location	Ubiquitous.

Background

Beclin-1, also known as also known as ATG6 or VPS30 is a protein that in humans is encoded by the BECN1 gene. Beclin-1 and its binding partner class III phosphoinositide 3-kinase (PI3K), also named Vps34, are required for the initiation of the formation of the autophagosome in autophagy. This gene participates in the regulation of autophagy and has an important role in development, tumorigenesis, and neurodegeneration. Schizophrenia is associated with low levels of Beclin-1 in the hippocampus of the affected which causes diminished autophagywhich in turn results in increased neuronal cell death. It has been found that beclin-1 can promote autophagy in autophagy-defective yeast with a targeted disruption of apg6/vps30, and in human MCF7 breast carcinoma cells.

Images

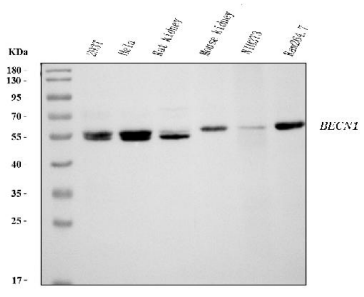


Figure 1. Western blot analysis of Beclin 1 using anti-Beclin 1 antibody (M00327-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human 293T 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 (Catalog # M00327-2) at 0.5 µg/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 (Catalog # EK1001) 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.

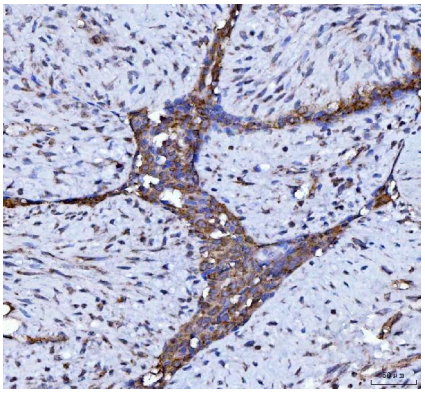


Figure 2. IHC analysis of Beclin 1 using anti-Beclin 1 antibody (M00327-2).
Beclin 1 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Beclin 1 Antibody (M00327-2) 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 (Catalog # SV0001) with DAB as the chromogen.

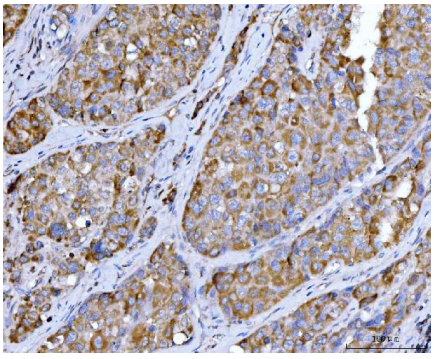


Figure 3. IHC analysis of Beclin 1 using anti-Beclin 1 antibody (M00327-2).
Beclin 1 was detected in a paraffin-embedded section of human hepatocellular carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Beclin 1 Antibody (M00327-2) 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 (Catalog # SV0001) with DAB as the chromogen.

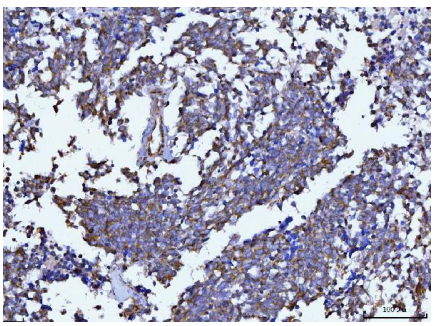
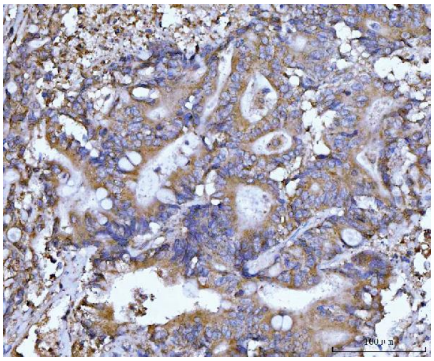


Figure 4. IHC analysis of Beclin 1 using anti-Beclin 1 antibody (M00327-2).
Beclin 1 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Beclin 1 Antibody (M00327-2) 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 (Catalog # SV0001) with DAB as the chromogen.

Figure 5. IHC analysis of Beclin 1 using anti-Beclin 1



antibody (M00327-2).
Beclin 1 was detected in a paraffin-embedded section of human colonic adenoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, 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 (M00327-2) 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 (Catalog # SV0001) with DAB as the chromogen.

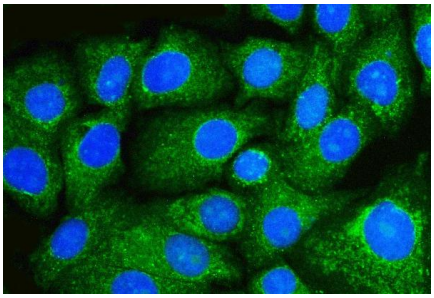


Figure 6. IF analysis of Beclin 1 using anti-Beclin 1 antibody (M00327-2).
Beclin 1 was detected in an immunocytochemical section of HepG2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Beclin 1 Antibody (M00327-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

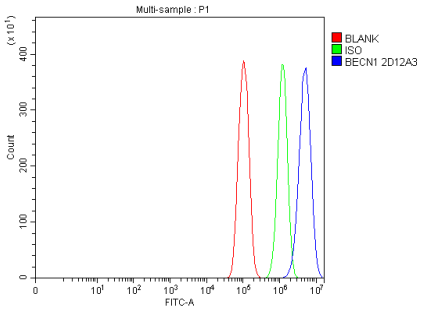


Figure 7. Flow Cytometry analysis of PC-3 cells using anti-Beclin 1 antibody (M00327-2).
Overlay histogram showing PC-3 cells stained with M00327-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Beclin 1 Antibody (M00327-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.