10320 Camino Santa Fe, Suite G
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Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3)

Catalog # ABO15049

Specification

Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) - Product Information

Application	**WB, IHC, FC**
Primary Accession	Q00610
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) - Additional Information

Gene ID 1213

Other Names
Clathrin heavy chain 1, Clathrin heavy chain on chromosome 17, CLH-17, CLH1

Calculated MW
180 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen
E.coli-derived human Clathrin heavy chain/CLTC recombinant protein (Position: R967-Q1668).

Purification
Immunogen affinity purified.

Storage	**Store 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 freeze-thaw cycles.**

Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) - Protein Information

Name CLH1

Function
Clathrin is the major protein of the polyhedral coat of coated pits and vesicles. Two different adapter protein complexes link the clathrin lattice either to the plasma membrane or to the trans-Golgi network. Acts as a component of the TACC3/ch-TOG/clathrin complex proposed to contribute to stabilization of kinetochore fibers of the mitotic spindle by acting as inter-microtubule bridge (PubMed:15858577, PubMed:16968737, PubMed:21297582). The TACC3/ch-TOG/clathrin complex is required for the maintenance of kinetochore fiber tension (PubMed:23532825). Plays a role in early autophagosome formation (PubMed:20639872). Interaction with DNAJC6 mediates the recruitment of HSPA8 to the clathrin lattice and creates local destabilization of the lattice promoting uncoating (By similarity).

Cellular Location
Cytoplasmic vesicle membrane; Peripheral membrane protein; Cytoplasmic side. Membrane, coated pit; Peripheral membrane protein; Cytoplasmic side. Melanosome. Cytoplasm, cytoskeleton, spindle. Note=Cytoplasmic face of coated pits and vesicles. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. In complex with TACC3 and CKAP5 (forming the TACC3/ch-TOG/clathrin complex) localized to inter-microtubule bridges in mitotic spindles.

Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) - Images

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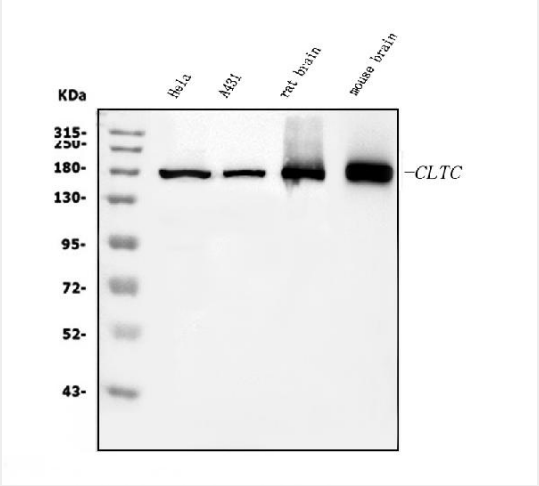

Figure 1. Western blot analysis of Clathrin heavy chain/CLTC using anti-Clathrin heavy chain/CLTC antibody (M03134-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human A431 whole cell lysates,
Lane 3: rat brain tissue lysates,
Lane 4: mouse brain tissue lysates.
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 mouse anti-Clathrin heavy chain/CLTC antigen affinity purified monoclonal antibody (Catalog # M03134-2) 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 Clathrin heavy chain/CLTC at approximately 180KD. The expected band size for Clathrin heavy chain/CLTC is at 180KD.

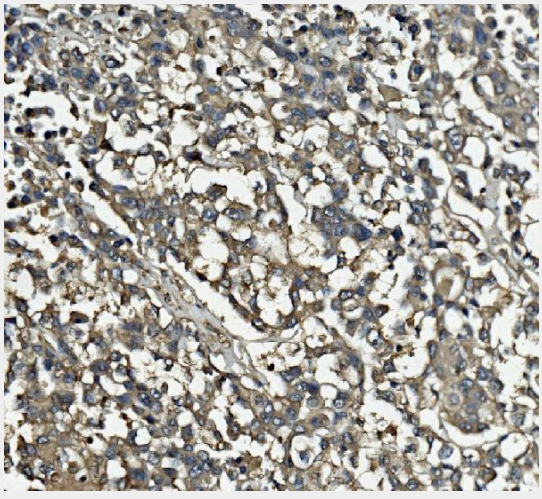

Figure 2. IHC analysis of Clathrin heavy chain/CLTC using anti-Clathrin heavy chain/CLTC antibody (M03134-2).
Clathrin heavy chain/CLTC was detected in paraffin-embedded section of human pancreatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Clathrin heavy chain/CLTC Antibody (M03134-2)

overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

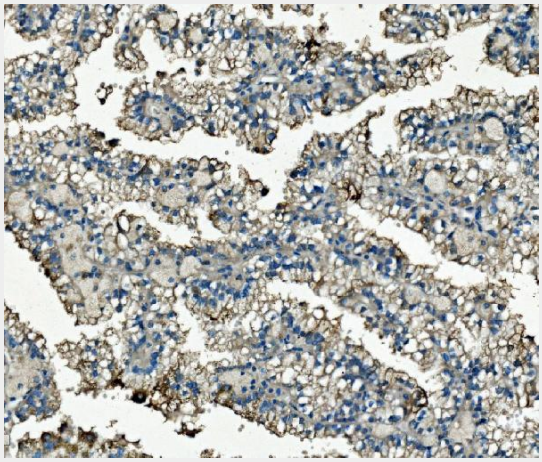

Figure 3. IHC analysis of Clathrin heavy chain/CLTC using anti-Clathrin heavy chain/CLTC antibody (M03134-2).
Clathrin heavy chain/CLTC was detected in paraffin-embedded section of human renal carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Clathrin heavy chain/CLTC Antibody (M03134-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

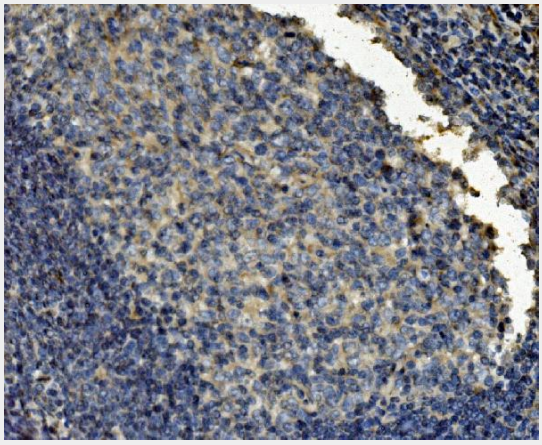

Figure 4. IHC analysis of Clathrin heavy chain/CLTC using anti-Clathrin heavy chain/CLTC antibody (M03134-2).
Clathrin heavy chain/CLTC was detected in paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Clathrin heavy chain/CLTC Antibody (M03134-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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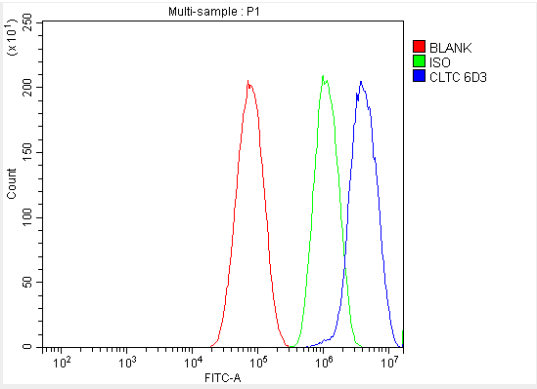

Figure 5. Flow Cytometry analysis of HepG2 cells using anti-Clathrin heavy chain/CLTC antibody (M03134-2).
Overlay histogram showing HepG2 cells stained with M03134-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Clathrin heavy chain/CLTC Antibody (M03134-2, 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.

Anti-Clathrin heavy chain/CLTC Antibody Picoband™ (monoclonal, 6D3) - Background

Clathrin heavy chain 1 is a protein that in humans is encoded by the CLTC gene. Clathrin is a major protein component of the cytoplasmic face of intracellular organelles, called coated vesicles and coated pits. These specialized organelles are involved in the intracellular trafficking of receptors and endocytosis of a variety of macromolecules. The basic subunit of the clathrin coat is composed of three heavy chains and three light chains.