10320 Camino Santa Fe, Suite G
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Anti-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11)

Catalog # ABO14990

Specification

Anti-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) - Product Information

Application	**WB, IHC, FC**
Primary Accession	Q9UKG1
Host	**Mouse**
Isotype	**Mouse IgG2a**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) . 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-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) - Additional Information

Gene ID 26060

Other Names
DCC-interacting protein 13-alpha, Dip13-alpha, Adapter protein containing PH domain, PTB domain and leucine zipper motif 1, APPL1 (HGNC:24035)

Calculated MW
85 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 APPL/APPL1 recombinant protein (Position: K91-R668).

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-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) - Protein Information

Name APPL1 (HGNC:24035)

Function
Multifunctional adapter protein that binds to various membrane receptors, nuclear factors and signaling proteins to regulate many processes, such as cell proliferation, immune response, endosomal trafficking and cell metabolism (PubMed:10490823, PubMed:15016378, PubMed:19661063, PubMed:26073777, PubMed:26583432). Regulates signaling pathway leading to cell proliferation through interaction with RAB5A and subunits of the NuRD/MeCP1 complex (PubMed:15016378). Functions as a positive regulator of innate immune response via activation of AKT1 signaling pathway by forming a complex with APPL1 and PIK3R1 (By similarity). Inhibits Fc-gamma receptor-mediated phagocytosis through PI3K/Akt signaling in macrophages (By similarity). Regulates TLR4 signaling in activated macrophages (By similarity). Involved in trafficking of the TGFBR1 from the endosomes to the nucleus via microtubules in a TRAF6-dependent manner (PubMed:26583432). Plays a role in cell metabolism by regulating adiponecting and insulin signaling pathways (PubMed:19661063, PubMed:24879834, PubMed:26073777). Required for fibroblast migration through HGF cell signaling (By similarity). Positive regulator of beta-catenin/TCF-dependent transcription through direct interaction with RUVBL2/reptin resulting in the relief of RUVBL2-mediated repression of beta-catenin/TCF target genes by modulating the interactions within the beta-catenin-reptin-HDAC complex (PubMed:19433865).

Cellular Location
Early endosome membrane; Peripheral membrane protein. Nucleus. Cytoplasm. Endosome. Cell projection, ruffle {ECO:0000250|UniProtKB:Q8K3H0}. Cytoplasmic vesicle, phagosome {ECO:0000250|UniProtKB:Q8K3H0}. Note=Early endosomal membrane-bound and nuclear. Translocated into the nucleus upon release from endosomal membranes following internalization of EGF

Tissue Location
High levels in heart, ovary, pancreas and skeletal muscle.

Anti-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) - 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-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) - Images

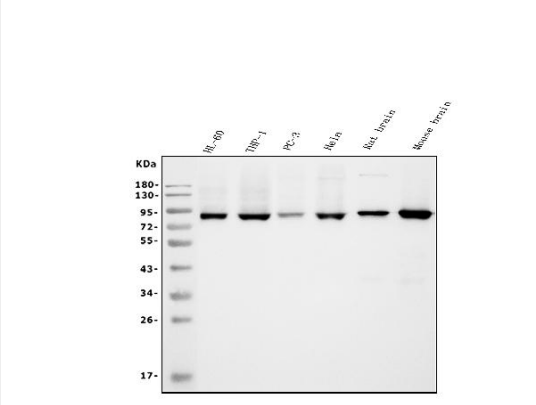

Figure 1. Western blot analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HL-60 whole cell lysates,
Lane 2: human THP-1 whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: human Hela whole cell lysates,
Lane 5: rat brain tissue lysates,
Lane 6: 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-APPL/APPL1 antigen affinity purified monoclonal antibody (Catalog # M02381) 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 APPL/APPL1 at approximately 85KD. The expected band size for APPL/APPL1 is at 85KD.

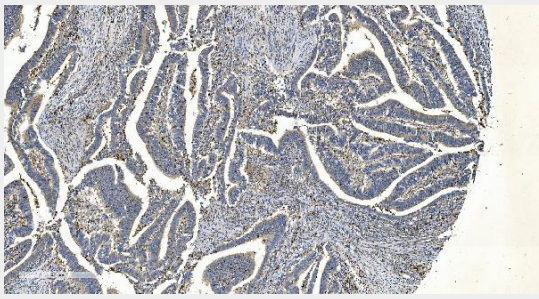

Figure 2. IHC analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
APPL/APPL1 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-APPL/APPL1 Antibody (M02381) 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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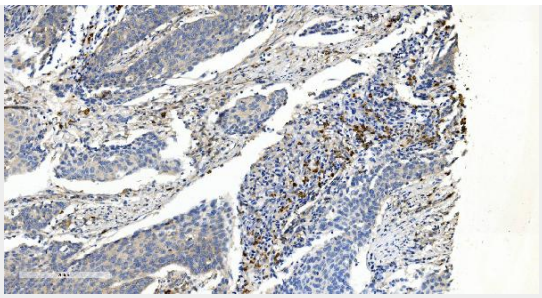

Figure 3. IHC analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
APPL/APPL1 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-APPL/APPL1 Antibody (M02381) 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.

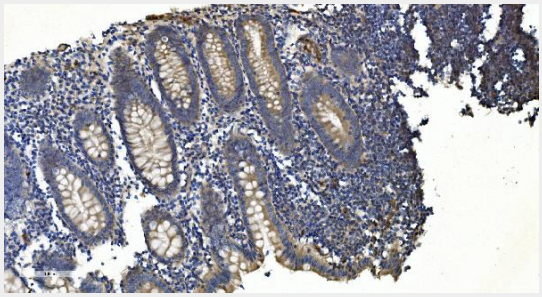

Figure 4. IHC analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
APPL/APPL1 was detected in paraffin-embedded section of human appendicitis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-APPL/APPL1 Antibody (M02381) 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.

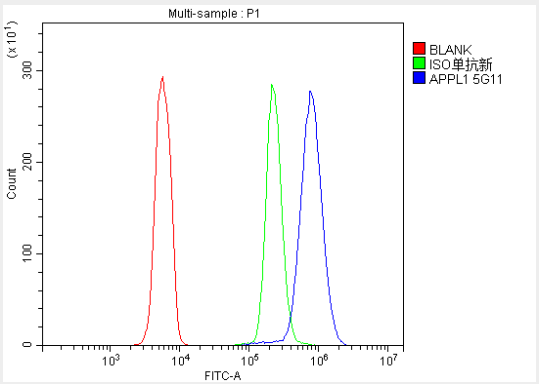

Figure 5. Flow Cytometry analysis of U-937 cells using anti-APPL/APPL1 antibody (M02381).
Overlay histogram showing U-937 cells stained with M02381 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-APPL/APPL1 Antibody (M02381, 1 µg/1x10^6 cells) for 30 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.

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Anti-APPL/APPL1 Antibody Picoband™ (monoclonal, 5G11) - Background

DCC-interacting protein 13-alpha (APPL1) is a protein that in humans is encoded by the APPL1 gene. The APPL1 gene is mapped to 3q21.1-p13.3. It is said to contain 709 amino acids and share 54% amino acid identity with APPL2. APPL is highly expressed in skeletal muscle, heart, ovary, and pancreas, tissues in which AKT2 mRNA is abundant. It has been regarded as an adaptor that may tether inactive AKT2 to the PI3K in the cytoplasm and thereby may expedite recruitment of AKT2 and PI3K to the cell membrane upon mitogenic stimulation.