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

Catalog # ABO14990

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.

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	79663
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 5G11
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.

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.

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.

Images

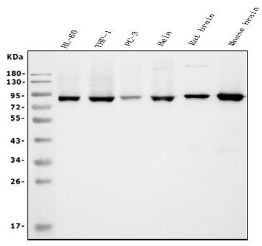


Figure 1. Western blot analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human 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.

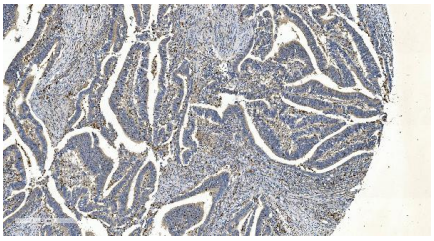


Figure 2. IHC analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
APPL/APPL1 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat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

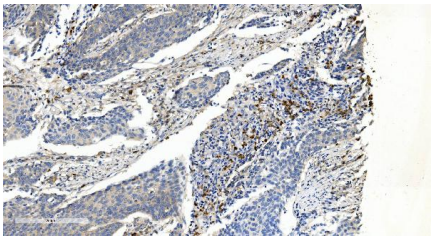


Figure 3. IHC analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
APPL/APPL1 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat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

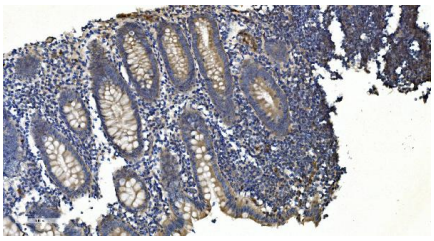
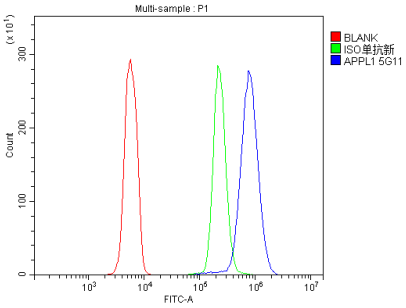


Figure 4. IHC analysis of APPL/APPL1 using anti-APPL/APPL1 antibody (M02381).
APPL/APPL1 was detected in paraffin-embedded section of human appendicitis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat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

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