10320 Camino Santa Fe, Suite G
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Anti-Rab11A Antibody Picoband™ (monoclonal, 4H9)

Catalog # ABO15077

Specification

Anti-Rab11A Antibody Picoband™ (monoclonal, 4H9) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P62491
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-Rab11A Antibody Picoband™ (monoclonal, 4H9) . 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 µg/ml.

Anti-Rab11A Antibody Picoband™ (monoclonal, 4H9) - Additional Information

Gene ID 8766

Other Names
Ras-related protein Rab-11A, Rab-11, 3.6.5.2, YL8, RAB11A (HGNC:9760)

Calculated MW
22 kDa KDa

Application Details
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, 1-3 µg/1x10^6 cells, Human, Mouse, Rat

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

Immunogen
A synthetic peptide corresponding to a sequence at the C-terminus of human Rab11A, identical to the related mouse and rat sequences.

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.

Anti-Rab11A Antibody Picoband™ (monoclonal, 4H9) - Protein Information

Name RAB11A (HGNC:9760)

Function
The small GTPases Rab are key regulators of intracellular membrane trafficking, from the formation of transport vesicles to their fusion with membranes. Rabs cycle between an inactive GDP-bound form and an active GTP-bound form that is able to recruit to membranes different set of downstream effectors directly responsible for vesicle formation, movement, tethering and fusion (PubMed:15601896, PubMed:15689490, PubMed:17462998, PubMed:19542231, PubMed:20026645, PubMed:20890297, PubMed:21282656). The small Rab GTPase RAB11A regulates endocytic recycling (PubMed:20026645). Forms a functional Rab11/FIP3/dynein complex that regulates the movement of peripheral sorting endosomes (SE) along microtubule tracks toward the microtubule organizing center/centrosome, generating the endosomal recycling compartment (ERC) (PubMed:20026645). Acts as a major regulator of membrane delivery during cytokinesis (PubMed:15601896). Together with MYO5B and RAB8A participates in epithelial cell polarization. Together with RAB3IP, RAB8A, the exocyst complex, PARD3, PRKCI, ANXA2, CDC42 and DNMBP promotes transcytosis of PODXL to the apical membrane initiation sites (AMIS), apical surface formation and lumenogenesis. Together with MYO5B participates in CFTR trafficking to the plasma membrane and TF (Transferrin) recycling in nonpolarized cells. Required in a complex with MYO5B and RAB11FIP2 for the transport of NPC1L1 to the plasma membrane. Participates in the sorting and basolateral transport of CDH1 from the Golgi apparatus to the plasma membrane. Regulates the recycling of FCGRT (receptor of Fc region of monomeric Ig G) to basolateral membranes. May also play a role in melanosome transport and release from melanocytes (PubMed:15689490, PubMed:17462998, PubMed:19542231, PubMed:20890297, PubMed:21282656). Promotes Rabin8/RAB3IP preciliary vesicular trafficking to mother centriole by forming a ciliary targeting complex containing Rab11, ASAP1, Rabin8/RAB3IP, RAB11FIP3 and ARF4, thereby regulating ciliogenesis initiation (PubMed:25673879, PubMed:31204173). On the contrary, upon LPAR1 receptor signaling pathway activation, interaction with phosphorylated WDR44 prevents Rab11-RAB3IP-RAB11FIP3 complex formation and cilia growth (PubMed:31204173). Participates in the export of a subset of neosynthesized proteins through a Rab8-Rab10-Rab11- endososomal dependent export route via interaction with WDR44 (PubMed:32344433).

Cellular Location
Cell membrane; Lipid-anchor. Endosome membrane. Recycling endosome membrane; Lipid-anchor. Cleavage furrow. Cytoplasmic vesicle, phagosome. Cytoplasmic vesicle membrane.

Golgi apparatus. Golgi apparatus, trans-Golgi network. Note=Localized to WDR44-positive endosomes and tubules (PubMed:32344433). Translocates with RAB11FIP2 from the vesicles of the endocytic recycling compartment (ERC) to the plasma membrane (PubMed:11994279). During interphase, localized in vesicles continuously moving from peripheral sorting endosomes towards the pericentrosomal ERC (PubMed:20026645). Localizes to the cleavage furrow (PubMed:15601896). Colocalizes with PARD3, PRKCI, EXOC5, OCLN, PODXL and RAB8A in apical membrane initiation sites (AMIS) during the generation of apical surface and lumenogenesis (PubMed:20890297) Recruited to phagosomes containing S.aureus or M.tuberculosis (PubMed:21255211). Localized to rhodopsin transport carriers when interacting with RAB11AFIP3 and ASAP1 in photoreceptors (PubMed:25673879).

Anti-Rab11A Antibody Picoband™ (monoclonal, 4H9) - 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-Rab11A Antibody Picoband™ (monoclonal, 4H9) - Images

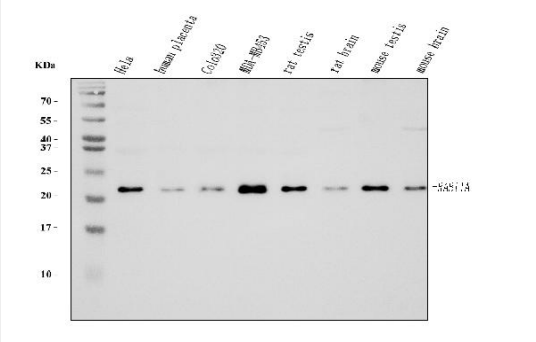

Figure 1. Western blot analysis of Rab11A using anti-Rab11A antibody (M01436-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human placenta tissue lysates,
Lane 3: human Colo320 lysates,
Lane 4: human MDA-MB453 whole cell lysates,
Lane 5: rat testis tissue lysates,
Lane 6: rat brain tissue lysates,
Lane 7: mouse testis tissue lysates,
Lane 8: mouse brain tissue 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-Rab11A antigen affinity purified monoclonal antibody (Catalog # M01436-2) 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 Rab11A at approximately 22 kDa. The expected band size for Rab11A is at 22 kDa.

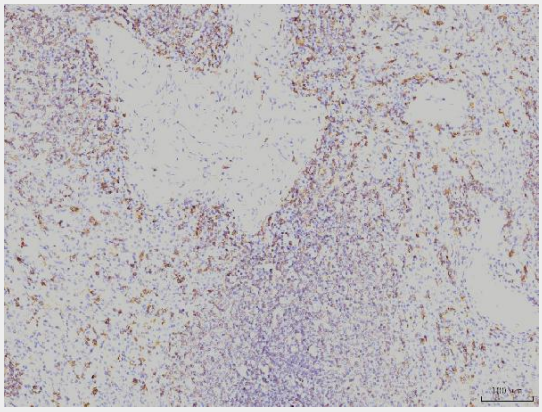

Figure 2. IHC analysis of Rab11A using anti-Rab11A antibody (M01436-2).
Rab11A was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Rab11A Antibody (M01436-2) 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.

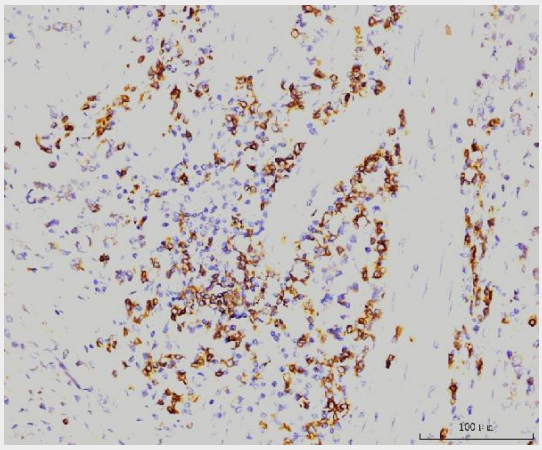

Figure 3. IHC analysis of Rab11A using anti-Rab11A antibody (M01436-2).
Rab11A was detected in a paraffin-embedded section of human gastric carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Rab11A Antibody (M01436-2) 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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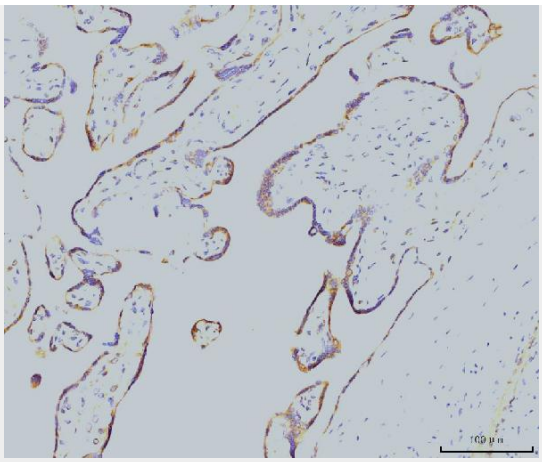

Figure 4. IHC analysis of Rab11A using anti-Rab11A antibody (M01436-2).
Rab11A was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Rab11A Antibody (M01436-2) 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.

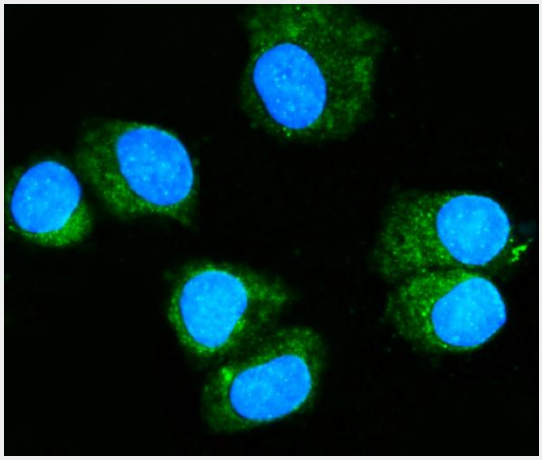

Figure 5. IF analysis of Rab11A using anti-Rab11A antibody (M01436-2).
Rab11A was detected in an immunocytochemical section of T-47D cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Rab11A Antibody (M01436-2) 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.

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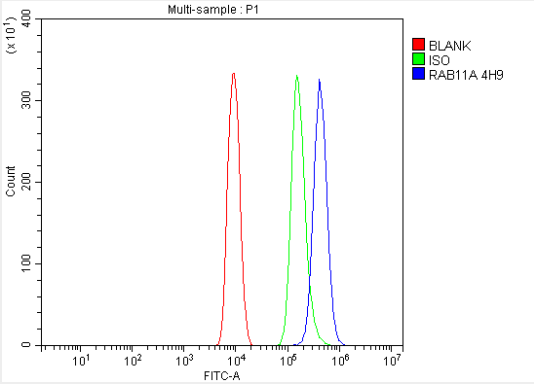

Figure 6. Flow Cytometry analysis of ANA-1 cells using anti-Rab11A antibody (M01436-2). Overlay histogram showing ANA-1 cells stained with M01436-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Rab11A Antibody (M01436-2, 1 µg/1x10^6 cells) for 30 min 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.

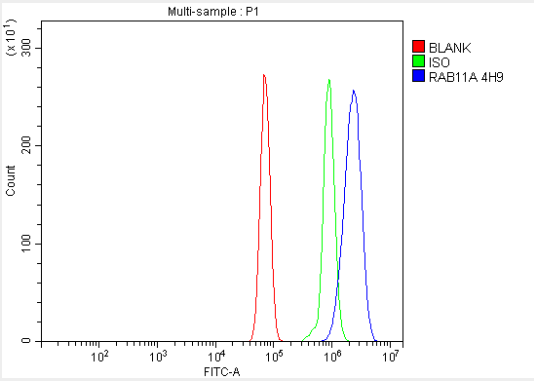

Figure 7. Flow Cytometry analysis of RH35 cells using anti-Rab11A antibody (M01436-2). Overlay histogram showing RH35 cells stained with M01436-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Rab11A Antibody (M01436-2, 1 µg/1x10^6 cells) for 30 min 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.

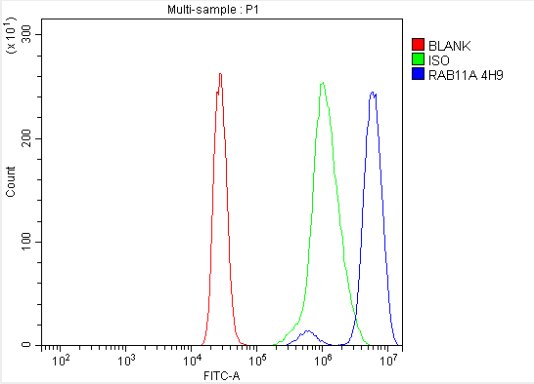

Figure 8. Flow Cytometry analysis of U937 cells using anti-Rab11A antibody (M01436-2). Overlay histogram showing U937 cells stained with M01436-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Rab11A Antibody (M01436-2, 1

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-Rab11A Antibody Picoband™ (monoclonal, 4H9) - Background

Ras-related protein Rab-11A is a protein that in humans is encoded by the RAB11A gene. The protein encoded by this gene belongs to the small GTPase superfamily, Rab family which plays essential roles in vesicle and granule targeting. It is mapped to 15q22.31. RAB11A is associated with both constitutive and regulated secretory pathways, and may be involved in protein transport. Additionally, RAB11A can control intracellular trafficking of the innate immune receptor TLR4, and thereby also receptor signaling. It has been shown to interact with RAB11FIP2, RAB11FIP4, and RAB11FIP1 and so on.