10320 Camino Santa Fe, Suite G
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Anti-RAB27A Antibody Picoband™ (monoclonal, 2F5)

Catalog # ABO14878

Specification

Anti-RAB27A Antibody Picoband™ (monoclonal, 2F5) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P51159
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-RAB27A Antibody Picoband™ (monoclonal, 2F5) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

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

Anti-RAB27A Antibody Picoband™ (monoclonal, 2F5) - Additional Information

Gene ID 5873

Other Names
Ras-related protein Rab-27A, Rab-27, 3.6.5.2, GTP-binding protein Ram, RAB27A, RAB27

Calculated MW
27 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml
 Immunocytochemistry/Immunofluorescence, 2 µg/ml
 Flow Cytometry, 1-3 µg/1x10^6 cells

Subcellular Localization
Lysosome. Late endosome. Membrane. Lipid-anchor. Melanosome.

Tissue Specificity
Found in all the examined tissues except in brain. Low expression was found in thymus, kidney, muscle and placenta. Detected in melanocytes, and in most tumor cell lines examined. Expressed in cytotoxic T-lymphocytes (CTL) and mast cells.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

Immunogen
E. coli-derived human RAB27A recombinant protein (Position: L98-K216).

Cross Reactivity
No cross-reactivity with other proteins.

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-RAB27A Antibody Picoband™ (monoclonal, 2F5) - Protein Information

Name RAB27A

Synonyms RAB27

Function
Small GTPase which cycles between active GTP-bound and inactive GDP-bound states. In its active state, binds to a variety of effector proteins to regulate homeostasis of late endocytic pathway, including endosomal positioning, maturation and secretion (PubMed:30771381). Plays a role in cytotoxic granule exocytosis in lymphocytes. Required for both granule maturation and granule docking and priming at the immunologic synapse.

Cellular Location
Membrane; Lipid-anchor. Melanosome. Late endosome. Lysosome. Note=Identified by mass spectrometry in melanosome fractions from stage I to stage IV (PubMed:12643545, PubMed:17081065). Localizes to endosomal exocytic vesicles (PubMed:17237785).

Tissue Location
Found in all the examined tissues except in brain. Low expression was found in thymus, kidney, muscle and placenta Detected in melanocytes, and in most tumor cell lines examined Expressed in cytotoxic T-lymphocytes (CTL) and mast cells

Anti-RAB27A Antibody Picoband™ (monoclonal, 2F5) - 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-RAB27A Antibody Picoband™ (monoclonal, 2F5) - Images

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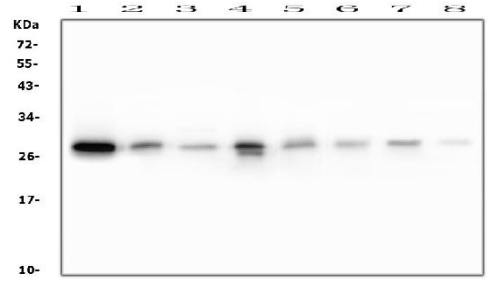

Figure 1. Western blot analysis of RAB27A using anti-RAB27A antibody (M01608).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human K562 whole cell lysates
Lane 2: human HepG2 whole cell lysates
Lane 3: human THP-1 whole cell lysates
Lane 4: human HT1080 whole cell lysates
Lane 5: human SW620 whole cell lysates
Lane 6: human PANC-1 whole cell lysates
Lane 7: rat RH35 whole cell lysates
Lane 8: mouse NIH/3T3 whole cell 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-RAB27A antigen affinity purified monoclonal antibody (Catalog # M01608) 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 RAB27A at approximately 27KD. The expected band size for RAB27A is at 27KD.

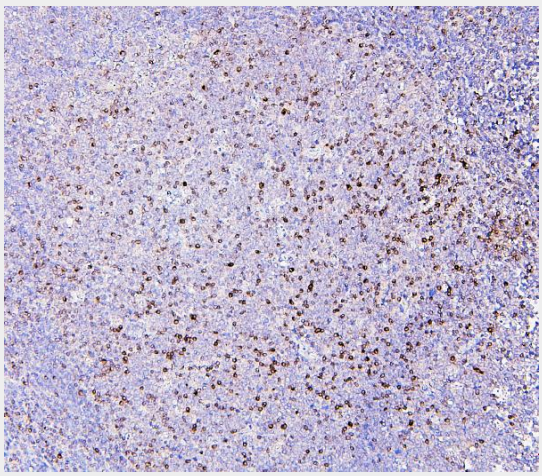

Figure 2. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RAB27A Antibody (M01608) 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.

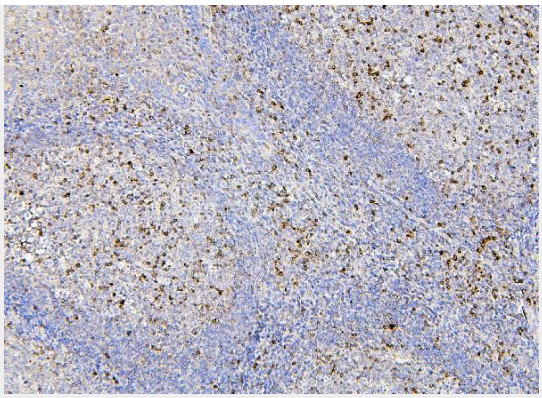

Figure 3. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RAB27A Antibody (M01608) 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.

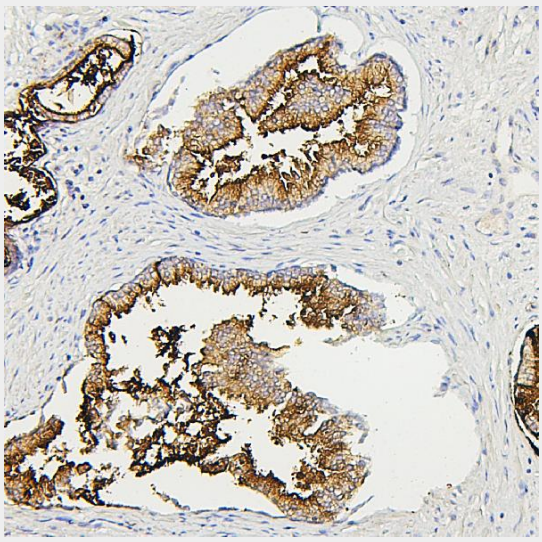

Figure 4. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of human prostatic cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RAB27A Antibody (M01608) 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.

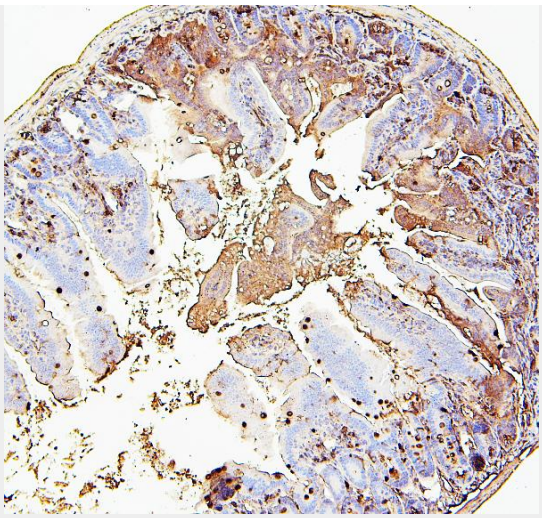

Figure 5. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of mouse intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RAB27A Antibody (M01608) 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.

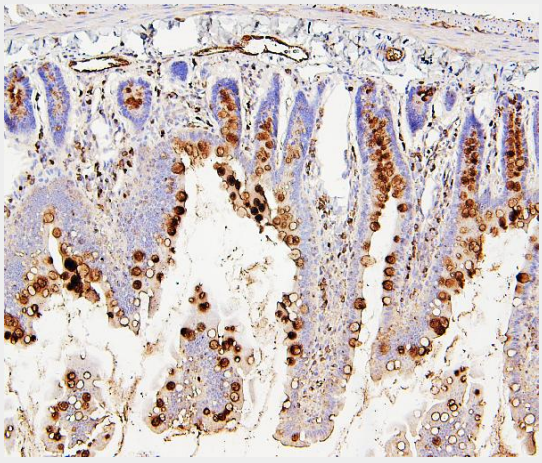

Figure 6. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of rat intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-RAB27A Antibody (M01608) 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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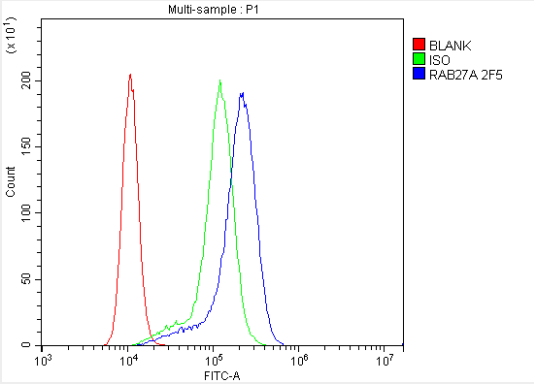

Figure 7. Flow Cytometry analysis of K562 cells using anti-RAB27A antibody (M01608).
Overlay histogram showing K562 cells stained with M01608 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAB27A Antibody (M01608,1 µg/1x10^6 cells) for 30 min at 20°C. 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.

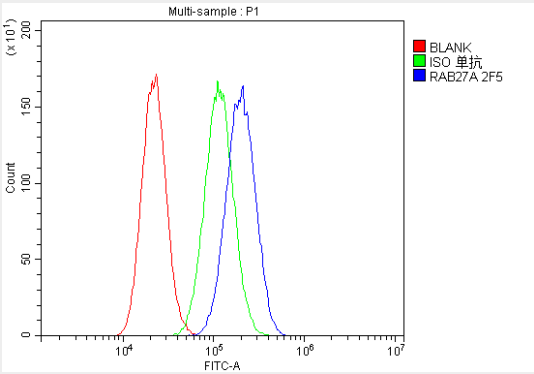

Figure 8. Flow Cytometry analysis of U87 cells using anti-RAB27A antibody (M01608).
Overlay histogram showing U87 cells stained with M01608 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAB27A Antibody (M01608,1 µg/1x10^6 cells) for 30 min at 20°C. 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.

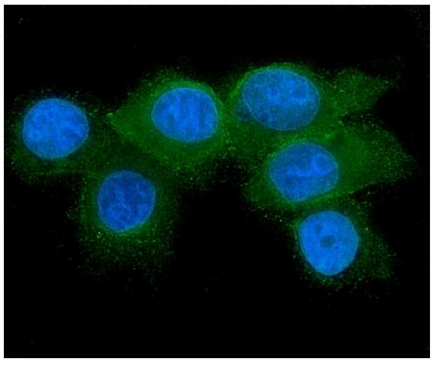

Figure 9. IF analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval

was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-RAB27A Antibody (M01608) 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.

Anti-RAB27A Antibody Picoband™ (monoclonal, 2F5) - Background

Ras-related protein Rab-27A is a protein that in humans is encoded by the RAB27A gene. The protein encoded by this gene belongs to the small GTPase superfamily, Rab family. The protein is membrane-bound and may be involved in protein transport and small GTPase mediated signal transduction. Mutations in this gene are associated with Griscelli syndrome type 2. Alternative splicing occurs at this locus and four transcript variants encoding the same protein have been identified.