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

Catalog # ABO14878

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 □g/ml.

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	24868
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/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.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 2F5
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.

Protein Information

Name	RAB27A (HGNC:9766)
Synonyms	RAB27
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 sets of downstream effectors directly responsible for vesicle formation, movement, tethering and fusion (PubMed:30771381). RAB27A regulates 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 (PubMed:18812475).
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

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.

Images

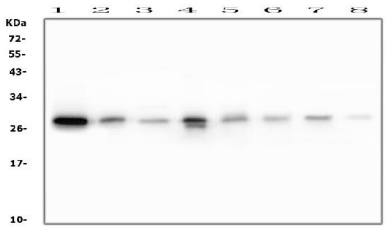


Figure 1. Western blot analysis of RAB27A using anti-RAB27A antibody (M01608).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human K562 whole 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.

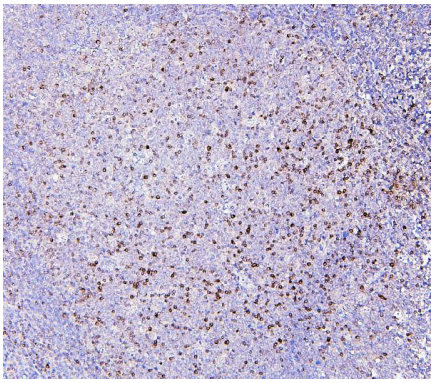


Figure 2. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum.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

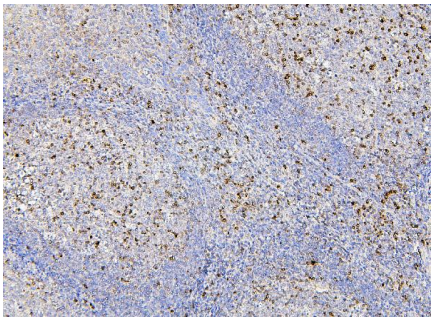


Figure 3. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum.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

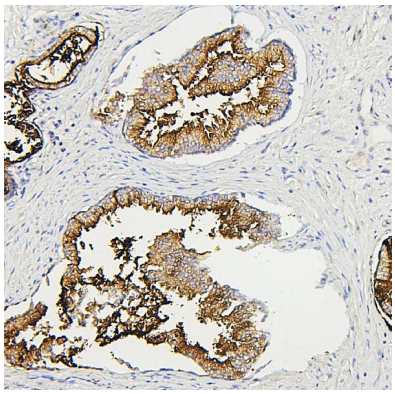
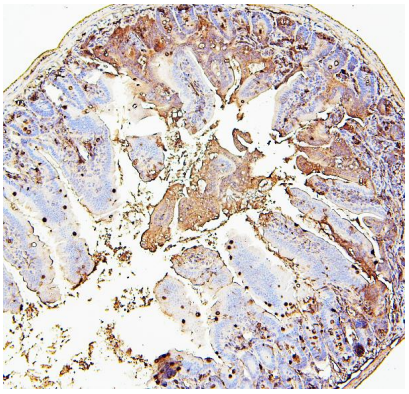


Figure 4. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of human prostatic cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 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.

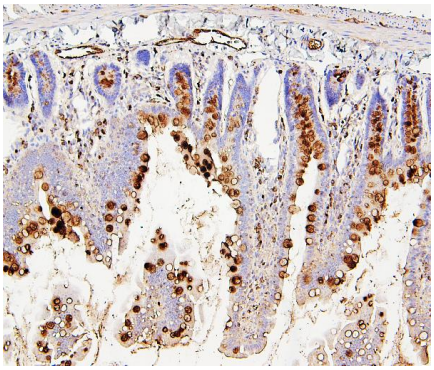


Figure 6. IHC analysis of RAB27A using anti-RAB27A antibody (M01608).
RAB27A was detected in paraffin-embedded section of rat intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat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

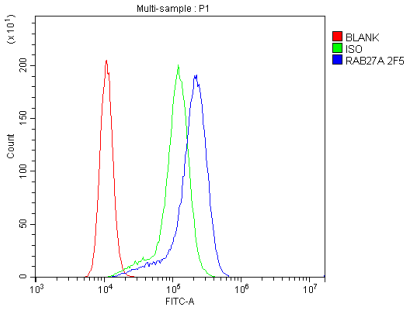


Figure 7. Flow Cytometry analysis of K562 cells using anti-RAB27A antibody (M01608).
Overlay histogram showing K562 cells stained with M01608 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAB27A Antibody (M01608,1 µg/1x10^6 cells) for 30 min at 20°C.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

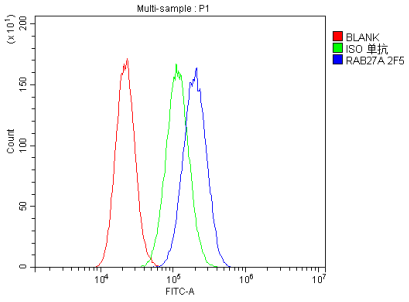
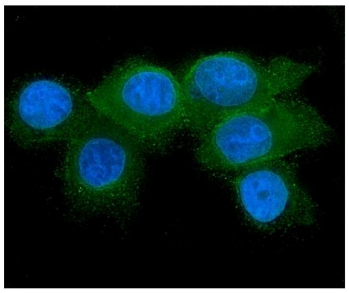


Figure 8. Flow Cytometry analysis of U87 cells using anti-RAB27A antibody (M01608).
Overlay histogram showing U87 cells stained with M01608 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAB27A Antibody (M01608,1 µg/1x10^6 cells) for 30 min at 20°C.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

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