

## Product Datasheet

### RAB27A Mouse Monoclonal Antibody (orb570319)

<b>Catalog Number</b>	orb570319
<b>Category</b>	Antibodies
<b>Description</b>	Anti-RAB27A Antibody (monoclonal, 2F5). Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Target</b>	Ras-related protein Rab-27A
<b>Clonality</b>	Monoclonal
<b>Species/Host</b>	Mouse
<b>Isotype</b>	Mouse IgG2b
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rat
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	500 µg/ml
<b>Buffer/Preservatives</b>	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
<b>Reconstitution</b>	Add 0.2ml of distilled water will yield a concentration of 500µg/ml.
<b>Purification</b>	Immunogen affinity purified.
<b>Immunogen</b>	E. coli-derived human RAB27A recombinant protein (Position: L98-K216).
<b>UniProt ID</b>	<b>P51159</b>
<b>MW</b>	27 kDa

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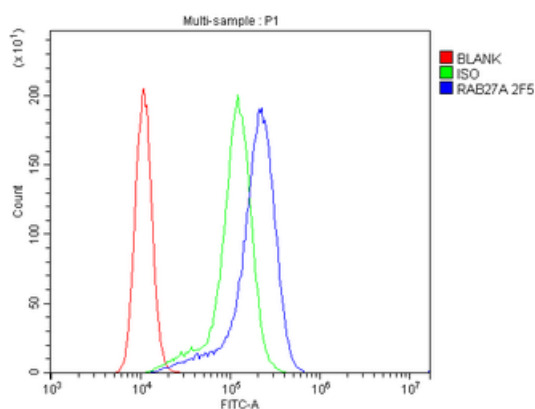
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<b>Tested applications</b>	FC, ICC, IF, IHC, WB
<b>Dilution range</b>	Western blot, 0.1-0.5µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml Immunocytochemistry/Immunofluorescence, 2µg/ml Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells
<b>Specificity</b>	No cross reactivity with other proteins.
<b>Cross Reactivity</b>	No cross-reactivity with other proteins.
<b>Antibody Type</b>	Primary Antibody
<b>Clone Number</b>	2F5
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Expiration Date</b>	12 months from date of receipt.



Flow Cytometry analysis of K562 cells using anti-RAB27A antibody. Overlay histogram showing K562 cells (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAB27A Antibody (1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (5-10 µg/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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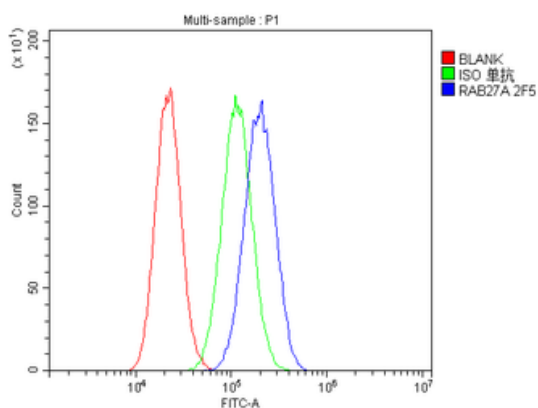
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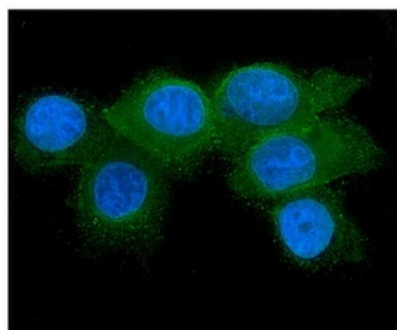
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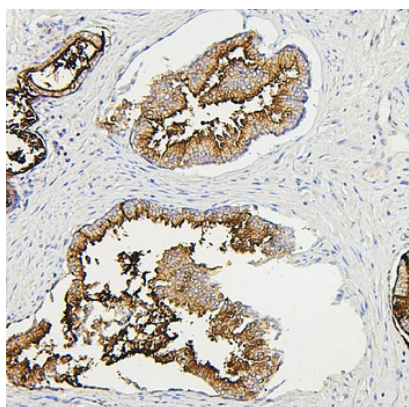
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Flow Cytometry analysis of U87 cells using anti-RAB27A antibody. Overlay histogram showing U87 cells (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAB27A Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



IF analysis of RAB27A using anti-RAB27A antibody. RAB27A was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2  $\mu\text{g}/\text{mL}$  mouse anti-RAB27A Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG 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.



IHC analysis of RAB27A using anti-RAB27A antibody. 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  $\mu\text{g}/\text{ml}$  mouse anti-RAB27A Antibody 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 Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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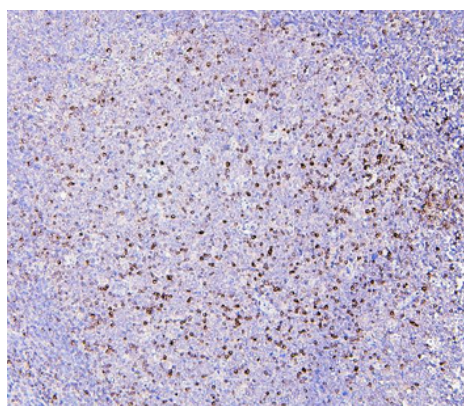
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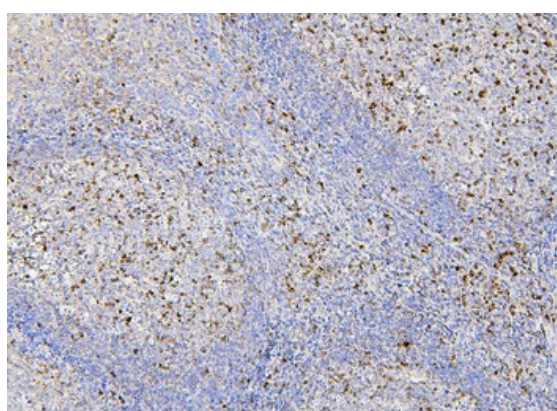
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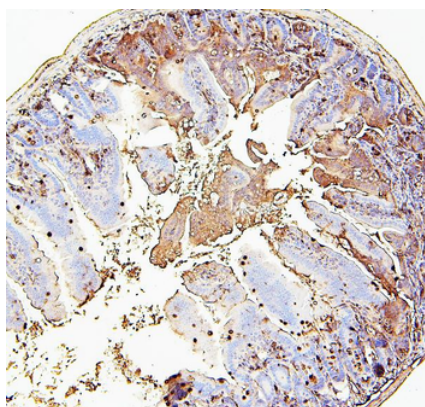
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IHC analysis of RAB27A using anti-RAB27A antibody. RAB27A was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml mouse anti-RAB27A Antibody 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 Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



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IHC analysis of RAB27A using anti-RAB27A antibody. 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  $\mu$ g/ml mouse anti-RAB27A Antibody 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 Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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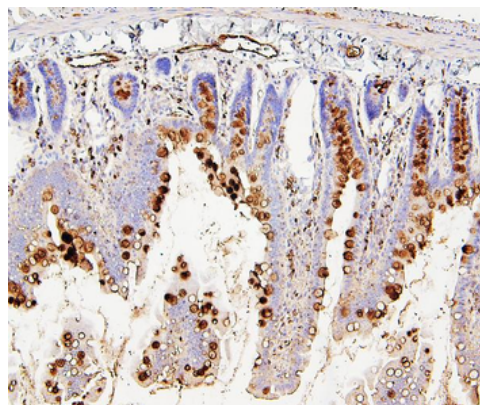
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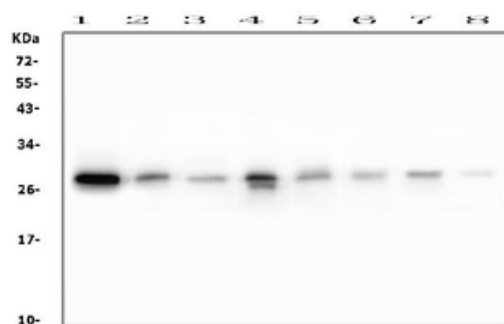
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IHC analysis of RAB27A using anti-RAB27A antibody. RAB27A was detected in paraffin-embedded section of rat intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue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



Western blot analysis of RAB27A using anti-RAB27A antibody. 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 50 µg 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 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 with Tanon 5200 system. A specific band was detected for RAB27A at approximately 27KD. The expected band size for RAB27A is at 27KD.

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