

## Product Datasheet

### Ran Antibody (monoclonal, 5D5) (orb570321)

<b>Catalog Number</b>	orb570321
<b>Description</b>	Anti-Ran Antibody (monoclonal, 5D5). Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Mouse
<b>Reactivity</b>	Human, Mouse, Rat
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	FC, ICC, IF, WB
<b>Immunogen</b>	E. coli-derived human Ran recombinant protein (Position: A2-L216). Human Ran shares 100% amino acid (aa) sequence identity with both mouse and rat Ran.
<b>Form/Appearance</b>	Lyophilized
<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>Application notes</b>	Western blot, 0.1-0.5µg/ml Immunocytochemistry/Immunofluorescence, 2µg/ml Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells. Add 0.2ml of distilled water will yield a concentration of 500µg/ml
<b>Isotype</b>	Mouse IgG2b
<b>Clonality</b>	Monoclonal
<b>Clone Number</b>	5D5
<b>Antibody Type</b>	Primary Antibody
<b>MW</b>	24 kDa

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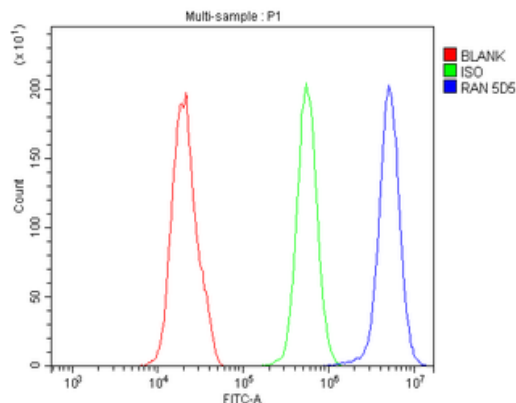
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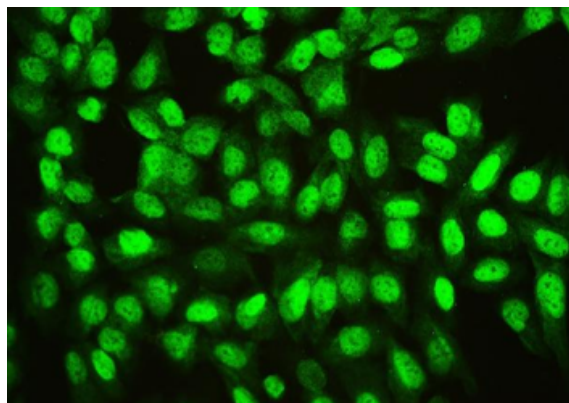
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**Uniprot ID****P62826****Expiration Date**

12 months from date of receipt.



Flow Cytometry analysis of PC-3 cells using anti-Ran antibody. Overlay histogram showing PC-3 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ran 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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



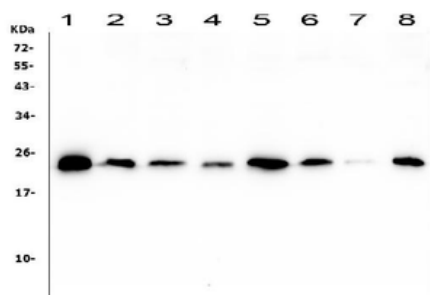
IF analysis of Ran using anti-Ran antibody. Ran was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2  $\mu\text{g}/\text{mL}$  mouse anti-Ran 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. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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Western blot analysis of Ran using anti-Ran antibody.

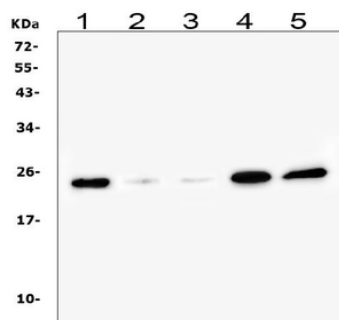
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 ug of sample under reducing conditions. Lane 1: human HL-60 whole cell lysates, Lane 2: human T-47D whole cell lysates, Lane 3: human A549 whole cell lysates, Lane 4: human U2OS whole cell lysates, Lane 5: human THP-1 whole cell lysates, Lane 6: human HepG2 whole cell lysates, Lane 7: human PANC-1 whole cell lysates, Lane 8: human SW620 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-Ran antigen affinity purified monoclonal antibody at 0.5  $\mu$ g/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 Ran at approximately 24KD. The expected band size for Ran is at 24KD.

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Western blot analysis of Ran using anti-Ran 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 ug of sample under reducing conditions. Lane 1: rat testis tissue lysates, Lane 2: mouse lung tissue lysates, Lane 3: mouse kidney tissue lysates, Lane 4: mouse testis tissue lysates, Lane 5: mouse Neuro-2a 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-Ran 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 Ran at approximately 24KD. The expected band size for Ran is at 24KD.

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