

## Product Datasheet

### Anti-RACK1 GNB2L1 Antibody (orb234289)

<b>Catalog Number</b>	orb234289
<b>Description</b>	Anti-RACK1 GNB2L1 Antibody. Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Rabbit
<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 RACK1 recombinant protein (Position: T2-R317). Human RACK1 shares 100% amino acid (aa) sequence identity with both mouse and rat RACK1.
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<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, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Human. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
<b>Isotype</b>	Rabbit IgG
<b>Clonality</b>	Polyclonal
<b>Antibody Type</b>	Primary Antibody

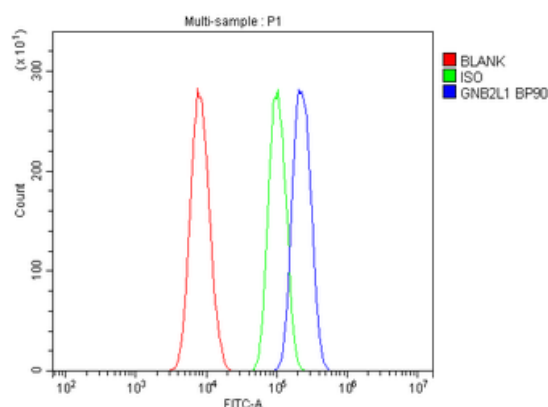
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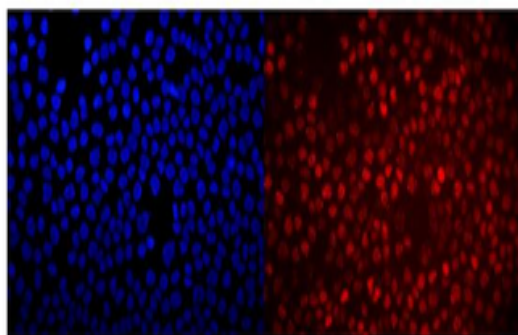
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<b>MW</b>	32 kDa
<b>Uniprot ID</b>	<b>P63244</b>
<b>Expiration Date</b>	12 months from date of receipt.



Flow Cytometry analysis of A431 cells using anti-RACK1 antibody. Overlay histogram showing A431 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 rabbit anti-RACK1 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit 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 rabbit 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.



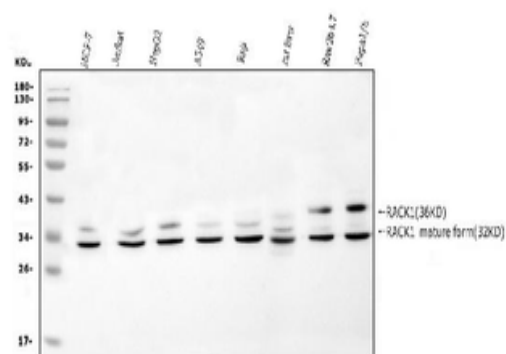
IF analysis of RACK1 using anti-RACK1 antibody. RACK1 was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2  $\mu\text{g}/\text{mL}$  rabbit anti-RACK1 Antibody overnight at 4°C. DyLight®550 Conjugated Goat Anti-Rabbit 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.

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Western blot analysis of RACK1 using anti-RACK1 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 30 ug of sample under reducing conditions. Lane 1: human MCF-7 whole cell lysates, Lane 2: human Jurkat whole cell lysates, Lane 3: human HepG2 whole cell lysates, Lane 4: human A549 whole cell lysates, Lane 5: human Raji whole cell lysates, Lane 6: rat liver tissue lysates, Lane 7: mouse RAW264.7 whole cell lysates, Lane 8: mouse HEPA1-6 whole cell 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 rabbit anti-RACK1 antigen affinity purified polyclonal 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-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 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 RACK1 at approximately 36 kDa and for RACK1 mature form at approximately 32 kDa. The expected band size for RACK1 is at 35 kDa.

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