Product Datasheet

Anti-Eph receptor B2/EPHB2 Antibody (orb623799)

Description	Anti-Eph receptor B2/EPHB2 Antibody. Tested in ELISA, Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Species/Host	Rabbit
Reactivity	Human, Mouse, Rat
Conjugation	Unconjugated
Tested Applications	ELISA, FC, ICC, IF, WB
Immunogen	E.coli-derived human Eph receptor B2/EPHB2 recombinant protein (Position: K278-K540).
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Storage	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.
Note	For research use only
Application notes	Western blot, 0.25-0.5µg/ml, Human Immunocytochemistry/Immunofluorescence, 2µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x106 cells, Human, Mouse, Rat ELISA, 0.1-0.5µg/ml, -. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
Isotype	Rabbit IgG
Clonality	Polyclonal
Antibody Type	Primary Antibody
MW	117 kDa
Uniprot ID	P29323

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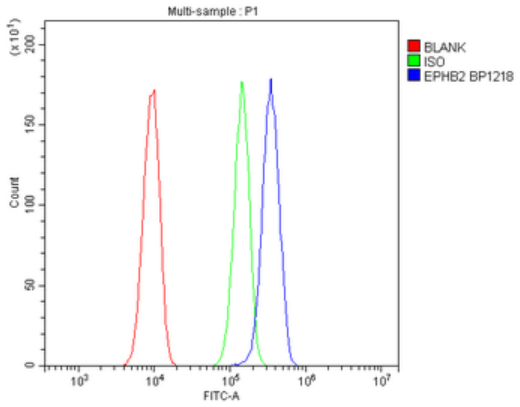
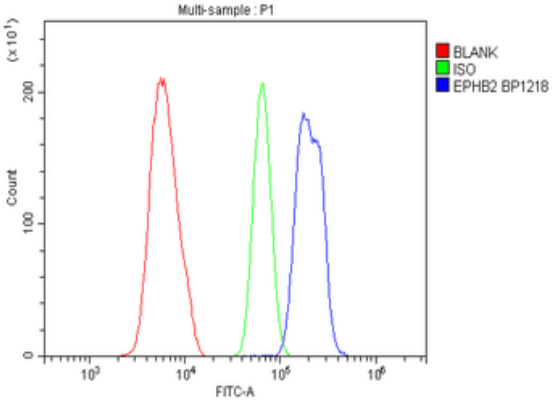
Expiration Date 12 months from date of receipt.



Flow Cytometry analysis of A549 cells using anti-EPHB2 antibody. Overlay histogram showing A549 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-EPHB2 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of Ana-1 cells using anti-EPHB2 antibody. Overlay histogram showing Ana-1 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-EPHB2 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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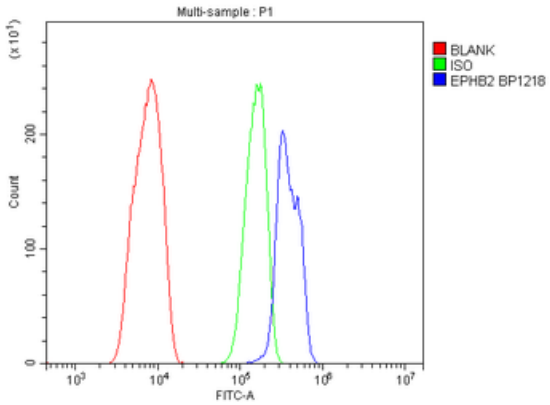
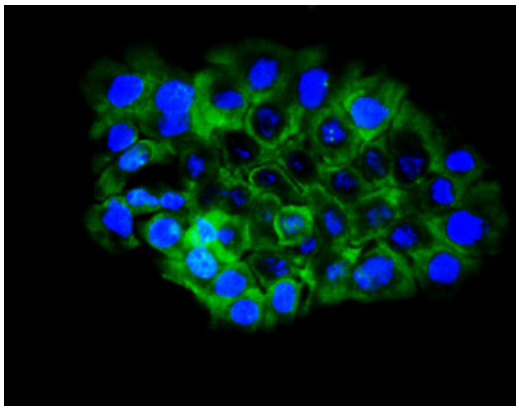


Flow Cytometry analysis of C6 cells using anti-EPHB2 antibody. Overlay histogram showing C6 cells (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-EPHB2 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



IF analysis of EPHB2 using anti-EPHB2 antibody. EPHB2 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 µg/mL rabbit anti-EPHB2 Antibody overnight at 4°C. DyLight®488 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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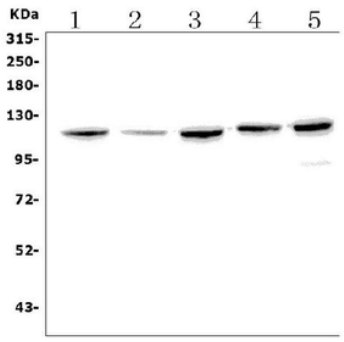

Western blot analysis of EPHB2 using anti-EPHB2 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 ug of sample under reducing conditions. Lane 1: human U-87MG whole cell lysates, Lane 2: human A549 whole cell lysates, Lane 3: human PC-3 whole cell lysates, Lane 4: human Caco-2 whole cell lysates, Lane 5: human K562 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-EPHB2 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 EPHB2 at approximately 117 KD. The expected band size for EPHB2 is at 117 KD.

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