Product Datasheet

Anti-BubR1/BUB1B Antibody (monoclonal, 5I7) (orb654276)

Description	Anti-BubR1/BUB1B Antibody (monoclonal, 5I7). Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Species/Host	Mouse
Reactivity	Human
Conjugation	Unconjugated
Tested Applications	FC, ICC, IF, IHC, WB
Immunogen	E.coli-derived human BubR1/BUB1B recombinant protein (Position: K26-E448).
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, 0.1-0.5µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml, Human Immunocytochemistry/Immunofluorescence, 2µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x106 cells, Human. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
Isotype	Mouse IgG1
Clonality	Monoclonal
Clone Number	5I7
Antibody Type	Primary Antibody

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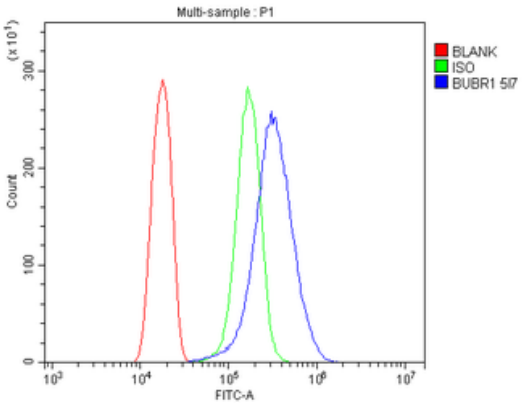
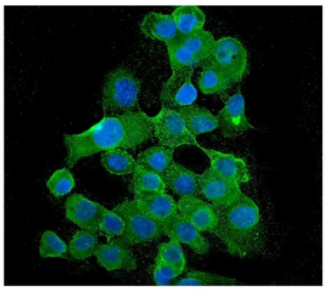
MW	130 kDa
Uniprot ID	**O60566**
Expiration Date	12 months from date of receipt.



Flow Cytometry analysis of Hela cells using anti-BubR1/BUB1B antibody. Overlay histogram showing Hela 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-BubR1/BUB1B Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody. BubR1/BUB1B 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 mouse anti-BubR1/BUB1B 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.

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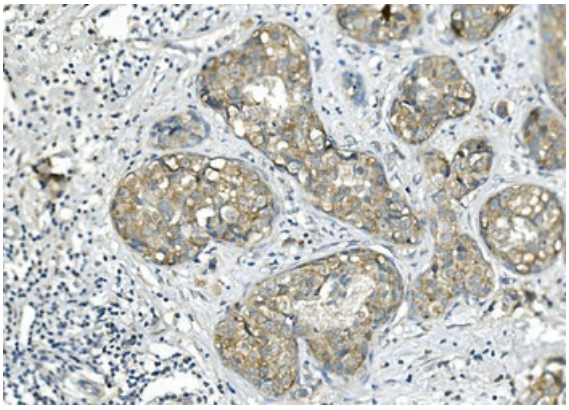
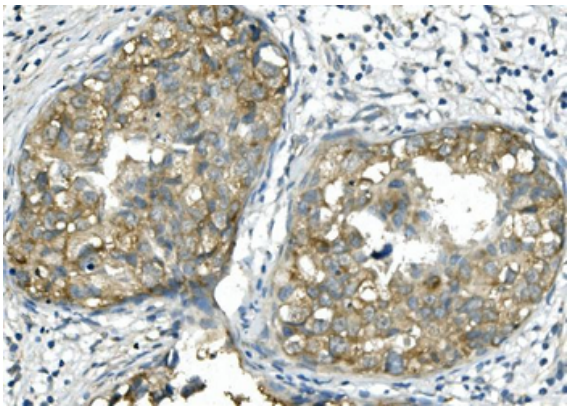
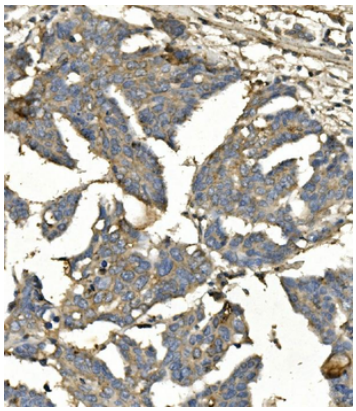


IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody. BubR1/BUB1B was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B 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 Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody. BubR1/BUB1B was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B 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 Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody. BubR1/BUB1B was detected in paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B 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 Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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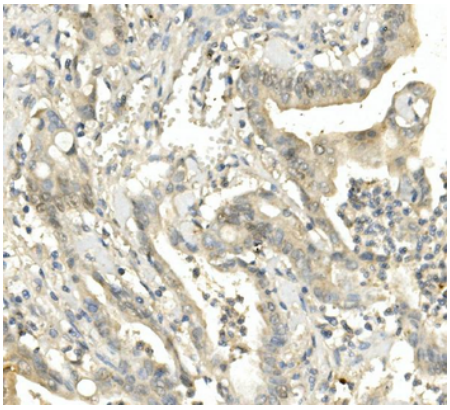
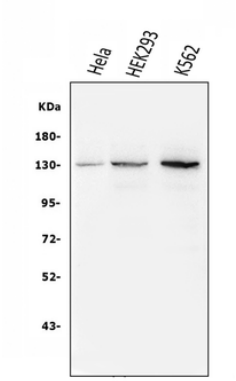


IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody. BubR1/BUB1B was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B 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 Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.



Western blot analysis of BubR1/BUB1B using anti-BubR1/BUB1B 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 Hela whole cell lysates; Lane 2: human HEK293 whole cell lysates; Lane 3: 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 mouse anti-BubR1/BUB1B 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 BubR1/BUB1B at approximately 130 KD. The expected band size for BubR1/BUB1B is at 120 KD.

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