10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

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

Catalog # ABO14942

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	O60566
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 5I7) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	701
Other Names	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta, 2.7.11.1, MAD3/BUB1-related protein kinase, hBUBR1, Mitotic checkpoint kinase MAD3L, Protein SSK1, BUB1B, BUBR1, MAD3L, SSK1
Calculated MW	119545
Application Details	Western blot, 0.1-0.5 □g/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Subcellular Localization	Centrosome. Nucleus. Cytoplasm. Kinetochore.
Tissue Specificity	Highly expressed in thymus followed by spleen. Preferentially expressed in tissues with a high mitotic index.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 5I7
Immunogen	E.coli-derived human BubR1/BUB1B recombinant protein (Position: K26-E448).
Purification	Immunogen affinity purified.

Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

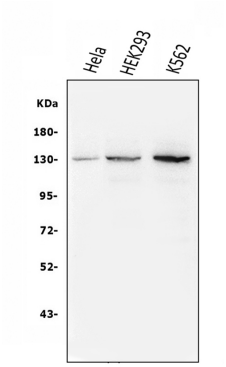
Name	BUB1B (HGNC:1149)
Function	Kinase which is a component of the mitotic checkpoint complex (MCC), which inhibits APC/C and delays chromosome segregation until all chromosomes are properly attached to the mitotic spindle (PubMed:27509861, PubMed:31201382). The MCC, which consists of CDC20, MAD2L1/MAD2, BUB1B/BUBR1 and BUB3, is a key player of the spindle assembly checkpoint (PubMed:11702782, PubMed:14706340, PubMed:15020684, PubMed:27509861, PubMed:31201382). The MCC-bound APC/C complex is inactive and this delays the metaphase/anaphase transition (PubMed:10477750, PubMed:27509861). When the spindle assembly checkpoint is silenced, the MCC-APC/C complex recruits the E2 enzyme UBCH10 and triggers the auto-ubiquitination of CDC20 in the MCC, thereby reactivating APC/C for transition into anaphase (PubMed:27509861). MCC also monitors kinetochore activities that depend on the kinetochore motor CENPE (PubMed:15020684). Required for kinetochore localization of CENPE (PubMed:15020684). Negatively regulates PLK1 activity in interphase cells and suppresses centrosome amplification (PubMed:19503101). May play a role for tumor suppression (By similarity).
Cellular Location	Cytoplasm. Nucleus. Chromosome, centromere, kinetochore. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome Note=Cytoplasmic in interphase cells. Associates with the kinetochores in early prophase. Kinetochore localization requires BUB1, PLK1 and KNL1.
Tissue Location	Highly expressed in thymus followed by spleen. Preferentially expressed in tissues with a high mitotic index

Background

Mitotic checkpoint serine/threonine-protein kinase BUB1 betais anenzymethat in humans is encoded by theBUB1Bgene. This gene encodes a kinase involved in spindle checkpoint function. The protein has been localized to the kinetochore and plays a role in the inhibition of the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation. Impaired spindle checkpoint function has been found in many forms of cancer.

Images

Figure 1. Western blot analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-2).
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 50ug 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 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-BubR1/BUB1B antigen affinity purified monoclonal antibody (Catalog # M01564-2) 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 (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for BubR1/BUB1B at approximately 130KD. The expected band size for BubR1/BUB1B is at 120KD.

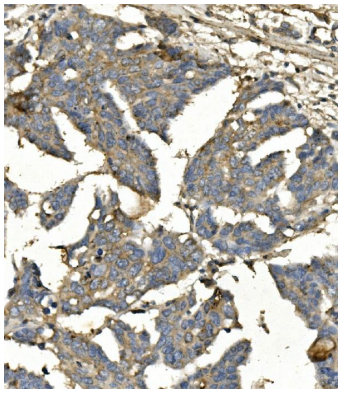


Figure 2. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-2). BubR1/BUB1B was detected in paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B Antibody (M01564-2) 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) (Catalog # SA1021) with DAB as the chromogen.

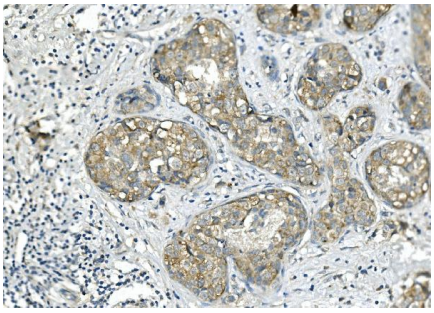


Figure 3. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-2). BubR1/BUB1B was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B Antibody (M01564-2) 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) (Catalog # SA1021) with DAB as the chromogen.

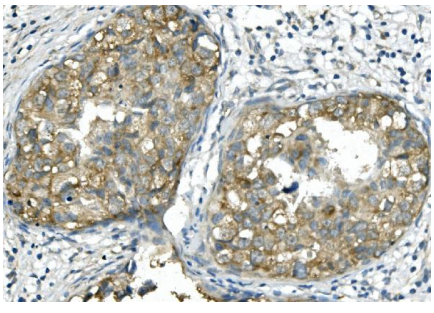
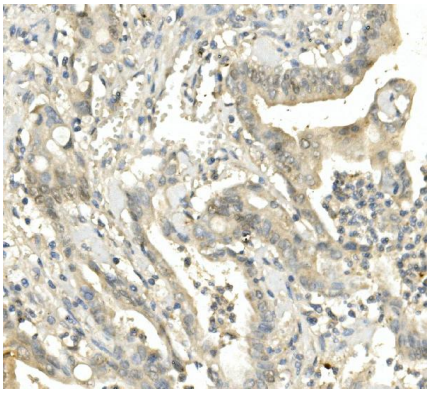


Figure 4. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-2). BubR1/BUB1B was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B Antibody (M01564-2) 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) (Catalog # SA1021) with DAB as the chromogen.

Figure 5. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-2). 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 (M01564-2) 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) (Catalog # SA1021) with DAB as the chromogen.

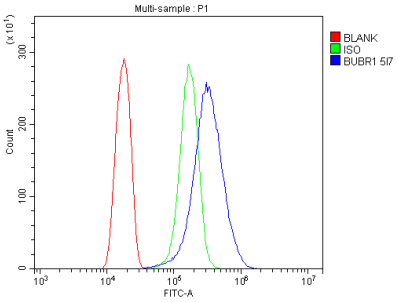


Figure 6. Flow Cytometry analysis of Hela cells using anti-BubR1/BUB1B antibody (M01564-2).
Overlay histogram showing Hela cells stained with M01564-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-BubR1/BUB1B Antibody (M01564-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 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 (Red line) was also used as a control.

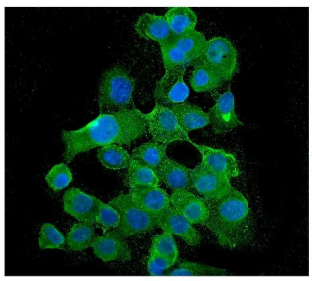


Figure 7. IF analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-2).
BubR1/BUB1B was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-BubR1/BUB1B Antibody (M01564-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.