10320 Camino Santa Fe, Suite G
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Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3)

Catalog # ABO14943

Specification

Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) - Product Information

Application	**WB, IHC, FC**
Primary Accession	O60566
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) - 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
130 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml, Human
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Rat
 Flow Cytometry, 1-3 µg/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.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

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.

Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) - Protein Information

Name BUB1B

Synonyms BUBR1, MAD3L, SSK1

Function
Essential component of the mitotic checkpoint. Required for normal mitosis progression. The mitotic checkpoint delays anaphase until all chromosomes are properly attached to the mitotic spindle. One of its checkpoint functions is to inhibit the activity of the anaphase- promoting complex/cyclosome (APC/C) by blocking the binding of CDC20 to APC/C, independently of its kinase activity. The other is to monitor kinetochore activities that depend on the kinetochore motor CENPE. Required for kinetochore localization of CENPE. Negatively regulates PLK1 activity in interphase cells and suppresses centrosome amplification. Also implicated in triggering apoptosis in polyploid cells that exit aberrantly from mitotic arrest. May play a role for tumor suppression.

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

Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) - Images

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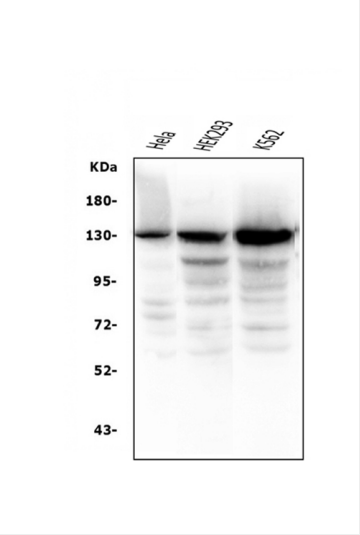

Figure 1. Western blot analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human 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 # M01564-3) 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.

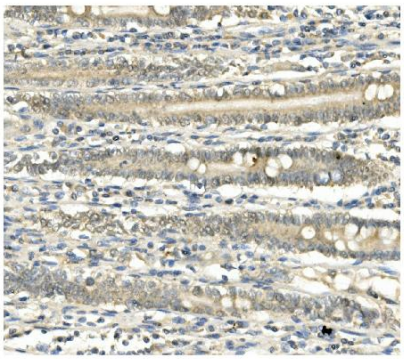

Figure 2. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-3).
BubR1/BUB1B was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with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

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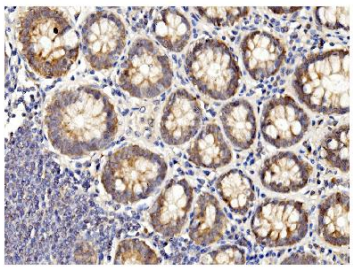

Figure 3. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-3).
BubR1/BUB1B was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with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

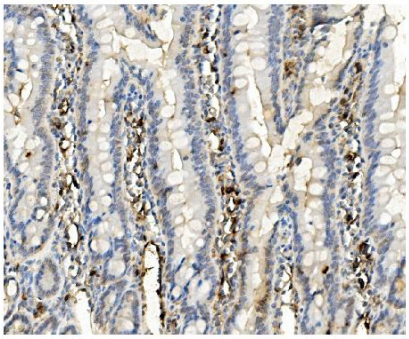

Figure 4. IHC analysis of BubR1/BUB1B using anti-BubR1/BUB1B antibody (M01564-3).
BubR1/BUB1B was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-BubR1/BUB1B Antibody (M01564-3) 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.

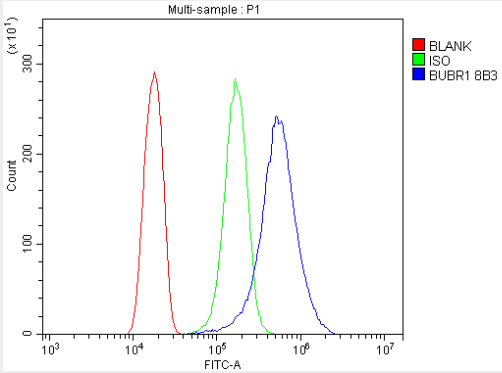

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Figure 5. Flow Cytometry analysis of Hela cells using anti-BubR1/BUB1B antibody (M01564-3). Overlay histogram showing Hela cells stained with M01564-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-BubR1/BUB1B Antibody (M01564-3, 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.

Anti-BubR1/BUB1B Antibody Picoband™ (monoclonal, 8B3) - 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.