abcam

Product datasheet

Anti-BubR1 (phospho T680) antibody [EPR19958] ab200061

Recombinant RabMAb

4 Images

Overview

Product name	Anti-BubR1 (phospho T680) antibody [EPR19958]
Description	Rabbit monoclonal [EPR19958] to BubR1 (phospho T680)
Host species	Rabbit
Tested applications	**Suitable for:** IP, Flow Cyt, ICC/IF, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human BubR1 aa 650-750 (phospho T680). The exact sequence is proprietary. Database link: O60566
Positive control	WB: HeLa whole cell lysate treated with 0.5 µM nocodazole for 24 hours. ICC/IF: HeLa cells. Flow Cyt: HeLa (human cervix adenocarcinoma) treated with 0.5 ng/ml nocodazole for 24 hours. IP: HeLa whole cell lysate treated with 0.5 µM nocodazole for 24 hours.
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19958

Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab200061** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

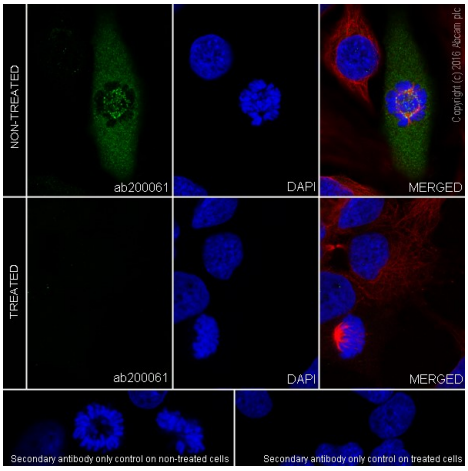
Application	Abreviews	Notes
IP		1/30.
Flow Cyt		1/500.
ICC/IF		1/100.
WB		1/2000. Detects a band of approximately 120 kDa (predicted molecular weight: 120 kDa).

Target

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.
Tissue specificity	Highly expressed in thymus followed by spleen. Preferentially expressed in tissues with a high mitotic index.
Involvement in disease	Note=Defects in BUB1B are associated with tumor formation. Defects in BUB1B are the cause of premature chromatid separation trait (PCS) [MIM:176430]. PCS consists of separate and splayed chromatids with discernible centromeres and involves all or most chromosomes of a metaphase. It is found in up to 2% of metaphases in cultured lymphocytes from approximately 40% of normal individuals. When PCS is present in 5% or more of cells, it is known as the heterozygous PCS trait and has no obvious phenotypic effect, although some have reported decreased fertility. Inheritance is autosomal dominant. Defects in BUB1B are the cause of mosaic variegated aneuploidy syndrome (MVA) [MIM:257300]. MVA is a severe autosomal recessive developmental disorder characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple different chromosomes and tissues. The proportion of aneuploid cells varies but is usually more than 25% and is substantially greater than in normal individuals. Affected individuals typically present with severe intrauterine growth retardation and microcephaly. Eye anomalies, mild dysmorphism, variable developmental delay, and a broad spectrum of additional congenital abnormalities and medical conditions may also occur. The risk of malignancy is high, with rhabdomyosarcoma, Wilms tumor and leukemia reported in several cases. MVA is caused by biallelic mutations in the BUB1B gene.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. BUB1 subfamily.

	Contains 1 BUB1 N-terminal domain. Contains 1 protein kinase domain.
Domain	The D-box targets the protein for rapid degradation by ubiquitin-dependent proteolysis during the transition from mitosis to interphase. The BUB1 N-terminal domain directs kinetochore localization and binding to BUB3.
Post-translational modifications	Proteolytically cleaved by caspase-3 in a cell cycle specific manner. The cleavage might be involved in the durability of the cell cycle delay. Caspase-3 cleavage is associated with abrogation of the mitotic checkpoint. The major site of cleavage is at Asp-610. Acetylation at Lys-250 regulates its degradation and timing in anaphase entry. Ubiquitinated. Degradated by the proteasome. Sumoylated by SUMO2 and SUMO3. The sumoylation mediates the association with CENPE at the kinetochore. Autophosphorylated in vitro. Intramolecular autophosphorylation is stimulated by CENPE. Phosphorylated during mitosis and hyperphosphorylated in mitotically arrested cells. Phosphorylation at Ser-670 and Ser-1043 occurs at kinetochores upon mitotic entry with dephosphorylation at the onset of anaphase.
Cellular localization	Cytoplasm. Nucleus. Chromosome > centromere > kinetochore. Cytoplasm > cytoskeleton > centrosome. Cytoplasmic in interphase cells. Associates with the kinetochores in early prophase. Kinetochore localization requires BUB1, PLK1 and CASC5.

Images



Immunocytochemistry/ Immunofluorescence - Anti-BubR1 (phospho T680) antibody [EPR19958] (ab200061)

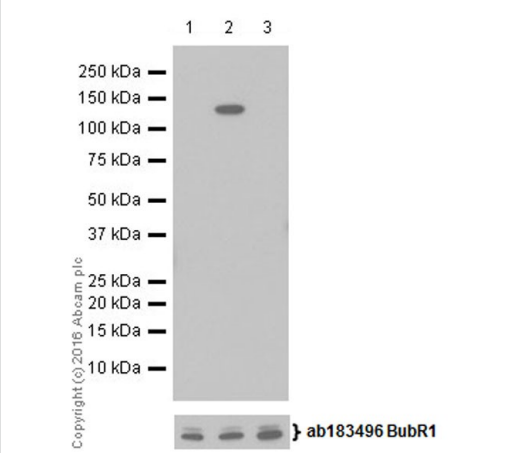
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling BubR1 (phospho T680) with ab200061 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing positive staining on M phase HeLa cells, and LP treatment completely blocked the staining. (PMID:11792804).

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/250 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 at 1/1000 dilution.



Western blot - Anti-BubR1 (phospho T680) antibody [EPR19958] (ab200061)

All lanes : Anti-BubR1 (phospho T680) antibody [EPR19958] (ab200061) at 1/2000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 0.5 µM nocodazole for 24 hours
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 0.5 µM nocodazole for 24 hours, then treated with FastAP Thermosensitive Alkaline Phosphatase for 1 hour

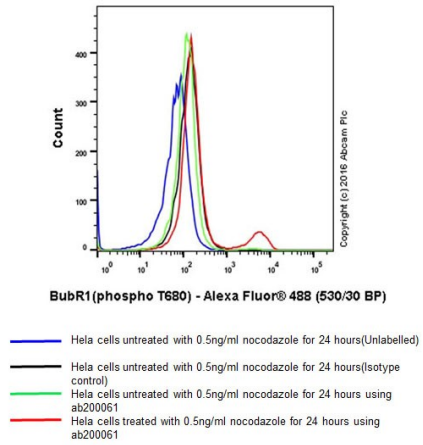
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 120 kDa
Observed band size: 120 kDa

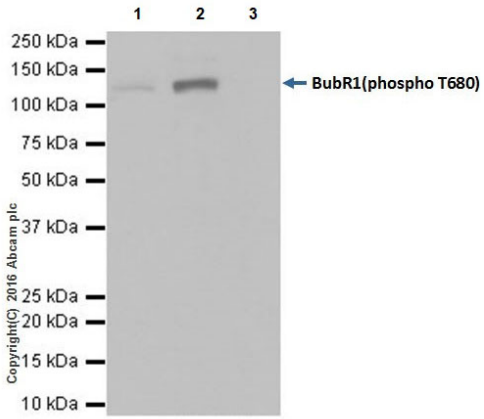
Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry - Anti-BubR1 (phospho T680) antibody [EPR19958] (ab200061)

Flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, untreated or treated with 0.5 ng/ml nocodazole for 24 hours, labeling BubR1 (phospho T680) with ab200061 at 1/500 dilution compared with a Rabbit IgG,monoclonal [EPR25A]-Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-BubR1 (phospho T680) antibody [EPR19958] (ab200061)

BubR1 (phospho T680) was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 0.5 µM nocodazole for 24 hours with ab200061 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab200061 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate treated with 0.5 µM nocodazole for 24 hours 10µg (Input).

Lane 2: ab200061 IP in HeLa whole cell lysate treated with 0.5 µM nocodazole for 24 hours.

Lane 3: Rabbit IgG,monoclonal [EPR25A]-Isotype Control (ab172730) instead of ab200061 in HeLa whole cell lysate treated with 0.5 µM nocodazole for 24 hours.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.

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