

Product datasheet

Anti-Bub1 antibody [EPR18947] ab195268

KO VALIDATED Recombinant **RabMAb**

[1 References](#) [7 Images](#)

Overview

Product name	Anti-Bub1 antibody [EPR18947]
Description	Rabbit monoclonal [EPR18947] to Bub1
Host species	Rabbit
Specificity	ab195268 shows stronger signal in mouse and rat testis tissues but weaker in the human testis.
Tested applications	Suitable for: WB, IHC-P, ICC
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment within Human Bub1 aa 1-200. The exact sequence is proprietary. Database link: O43683
Positive control	WB: HeLa, K562, U-2 OS, F9 and mESC whole cell lysates; Human testis and fetal liver lysates; Mouse and Rat testis lysates. IHC-P: Mouse and Rat testis tissues.
General notes	<p>Our in house IHC testing showed positive staining in testis tissue ONLY. Other tissues were negative.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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	EPR18947
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab195268** 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
WB		1/1000. Detects a band of approximately 122 kDa (predicted molecular weight: 122 kDa).
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Our in house IHC testing showed positive staining in testis tissue ONLY. Other tissues were negative.
ICC		1/100.

Target

Function	Serine/threonine-protein kinase that performs 2 crucial functions during mitosis: it is essential for spindle-assembly checkpoint signaling and for correct chromosome alignment. Has a key role in the assembly of checkpoint proteins at the kinetochore, being required for the subsequent localization of CENPF, BUB1B, CENPE and MAD2L1. Required for the kinetochore localization of PLK1. Plays an important role in defining SGOL1 localization and thereby affects sister chromatid cohesion. Acts as a substrate for anaphase-promoting complex or cyclosome (APC/C) in complex with its activator CDH1 (APC/C-Cdh1). Necessary for ensuring proper chromosome segregation and binding to BUB3 is essential for this function. Can regulate chromosome segregation in a kinetochore-independent manner. Can phosphorylate BUB3. The BUB1-BUB3 complex plays a role in the inhibition of APC/C when spindle-assembly checkpoint is activated and inhibits the ubiquitin ligase activity of APC/C by phosphorylating its activator CDC20. This complex can also phosphorylate MAD1L1. Kinase activity is essential for inhibition of APC/CCDC20 and for chromosome alignment but does not play a major role in the spindle-assembly checkpoint activity. Mediates cell death in response to chromosome missegregation and acts to suppress spontaneous tumorigenesis.
Tissue specificity	High expression in testis and thymus, less in colon, spleen, lung and small intestine. Expressed in fetal thymus, bone marrow, heart, liver, spleen and thymus. Expression is associated with cells/tissues with a high mitotic index.
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 KEN box is required for its ubiquitination and degradation. BUB1 N-terminal domain directs kinetochore localization and binding to BUB3.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR. Upon spindle-assembly checkpoint activation it is hyperphosphorylated and its kinase activity toward CDC20 is stimulated. Phosphorylation at Thr-609 is required for interaction with PLK1, phosphorylation at

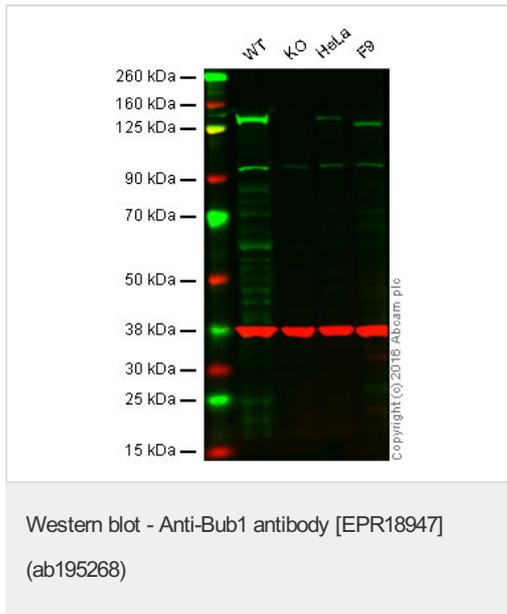
this site probably creates a binding site for the POLO-box domain of PLK1, thus enhancing the PLK1-BUB1 interaction.

Ubiquitinated and degraded during mitotic exit by APC/C-Cdh1.

Cellular localization

Nucleus. Chromosome > centromere > kinetochore. Nuclear in interphase cells. Accumulates gradually during G1 and S phase of the cell cycle, peaks at G2/M, and drops dramatically after mitosis. Localizes to the outer kinetochore. Kinetochore localization is required for normal mitotic timing and checkpoint response to spindle damage and occurs very early in prophase. AURKB, CASC5 and INCENP are required for kinetochore localization.

Images



Lane 1: Wild-type HAP1 cell lysate (20 µg)

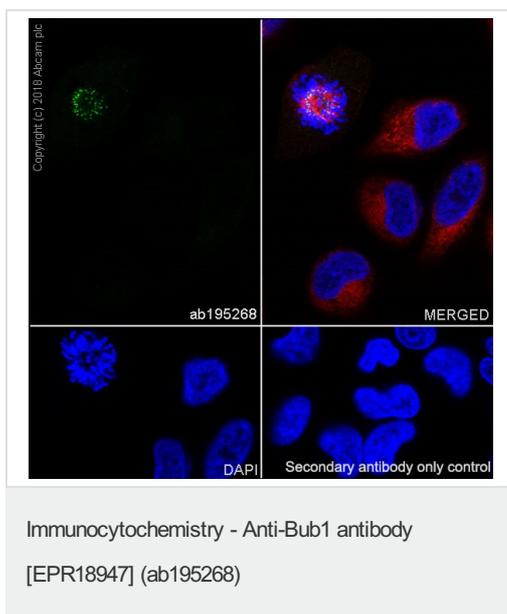
Lane 2: Bub1 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

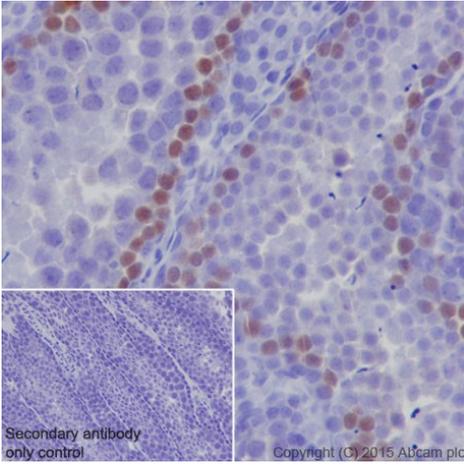
Lane 4: F9 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab195268 observed at 125 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab195268 was shown to recognize Bub1 when Bub1 knockout samples were used, along with additional cross-reactive bands. Wild-type and Bub1 knockout samples were subjected to SDS-PAGE. Ab195268 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10,000 dilution respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Ab195268 staining Bub1 in HeLa (human cervix adenocarcinoma epithelial cell) cells by Immunocytochemistry. Cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% TritonX-100. Samples were incubated with primary antibody at 1:100 dilution (7.8 µg/ml). Alexa Fluor® 594 Anti-alpha tubulin [DM1A] – Microtubule Marker (ab195889) was used as the counterstain antibody at 1:200 dilution (2.5 µg/ml). An AlexaFluor®488 Goat anti-Rabbit (ab150077) was used as a secondary antibody at 1:1000 dilution (2µg/ml). DAPI was used as a nuclear counterstain. Confocal image showing positive kinetochore staining in HeLa cells in M phase.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bub1 antibody [EPR18947] (ab195268)

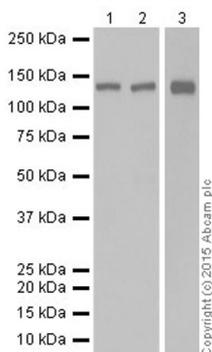
Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling Bub1 with ab195268 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nucleus staining on Rat testis is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Bub1 antibody [EPR18947] (ab195268)

All lanes : Anti-Bub1 antibody [EPR18947] (ab195268) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 3 : U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

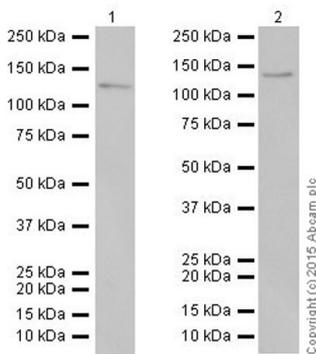
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 122 kDa

Observed band size: 122 kDa

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

Exposure time: Lane 1 & 2: 10 seconds; Lane 2: 3 minutes.



Western blot - Anti-Bub1 antibody [EPR18947]
(ab195268)

All lanes : Anti-Bub1 antibody [EPR18947] (ab195268) at 1/1000 dilution

Lane 1 : Human testis lysate

Lane 2 : Human fetal liver lysate

Lysates/proteins at 10 µg per lane.

Secondary

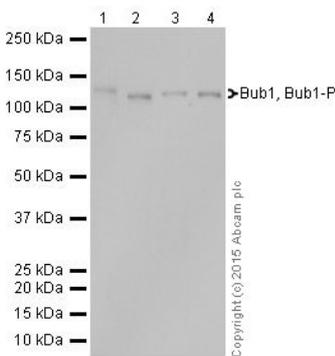
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 122 kDa

Observed band size: 122 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.



Western blot - Anti-Bub1 antibody [EPR18947]
(ab195268)

All lanes : Anti-Bub1 antibody [EPR18947] (ab195268) at 1/1000 dilution

Lane 1 : Mouse testis lysate

Lane 2 : Rat testis lysate

Lane 3 : F9 (Mouse embryonic testicular cancer cell line) whole cell lysate

Lane 4 : mESC (Mouse embryonic stem cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 122 kDa

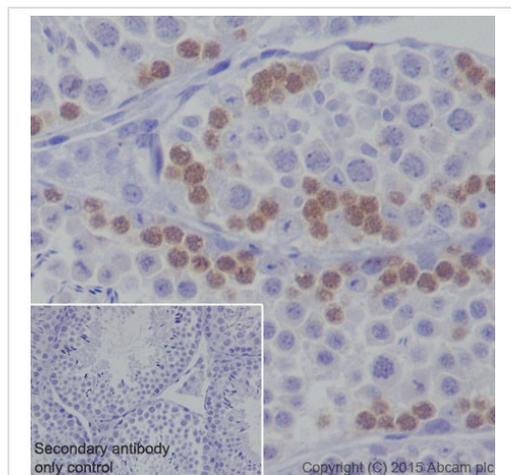
Observed band size: 122 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFD/MTBST.

The molecular weight observed is consistent with what has been described in the literature PMID:17189386

PMID:16864798



Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling Bub1 with ab195268 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nucleus staining on Mouse testis is observed.

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bub1 antibody [EPR18947] (ab195268)

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