

Anti-Bub1 antibody [EPR18947] ab195268

敲除验证
重组
RabMAb

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概述

产品名称	Anti-Bub1抗体[EPR18947]
描述	兔单克隆抗体[EPR18947] to Bub1
宿主	Rabbit
特异性	ab195268 shows stronger signal in mouse and rat testis tissues but weaker in the human testis.
经测试应用	适用于: WB, IHC-P, ICC/IF
种属反应性	与反应: Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
常规说明	<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>

性能

形式	Liquid
存放说明	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.
存储溶液	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR18947
同种型	IgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab195268于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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/IF		1/100.

靶标

功能	<p>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.</p>
组织特异性	<p>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.</p>
序列相似性	<p>Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. BUB1 subfamily. Contains 1 BUB1 N-terminal domain. Contains 1 protein kinase domain.</p>
结构域	<p>The KEN box is required for its ubiquitination and degradation. BUB1 N-terminal domain directs kinetochore localization and binding to BUB3.</p>
翻译后修饰	<p>Phosphorylated upon DNA damage, probably by ATM or ATR. Upon spindle-assembly checkpoint activation it is hyperphosphorylated and its kinase activity toward CDC20 is</p>

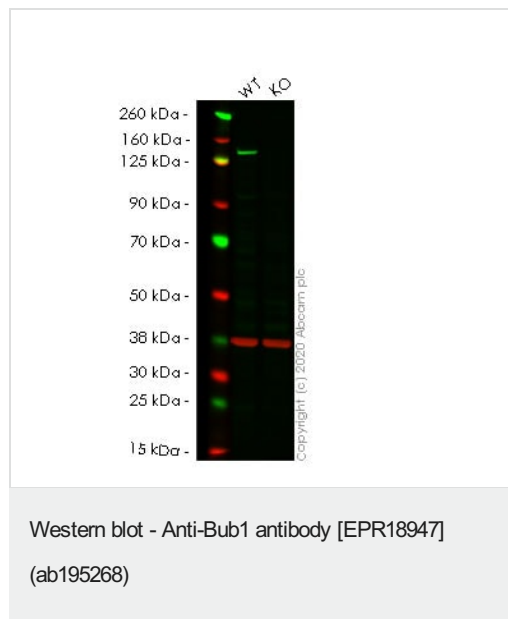
stimulated. Phosphorylation at Thr-609 is required for interaction with PLK1, phosphorylation at this site probably creates a binding site for the POLO-box domain of PLK1, thus enhancing the PLK1-BUB1 interaction.

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

细胞定位

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.

图片



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

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : BUB1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

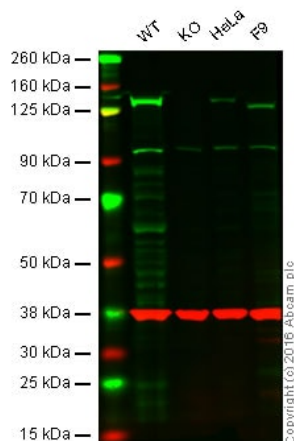
Performed under reducing conditions.

Predicted band size: 122 kDa

Observed band size: 125 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab195268 observed at 125 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

ab195268 was shown to react with Bub1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line [ab265145](#) (knockout cell lysate [ab257373](#)) was used. Wild-type HeLa and BUB1 knockout HeLa cell lysates were subjected to SDS-PAGE. ab195268 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



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

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

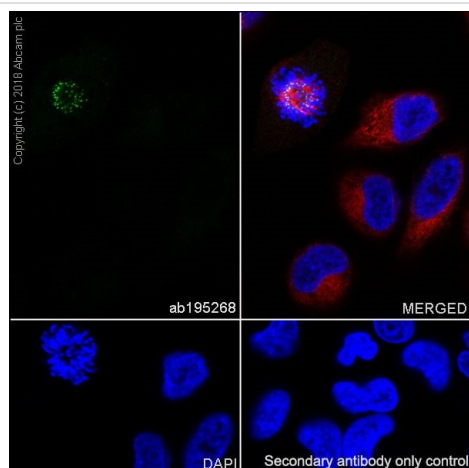
Lane 2: Bub1 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: F9 cell lysate (20 µg)

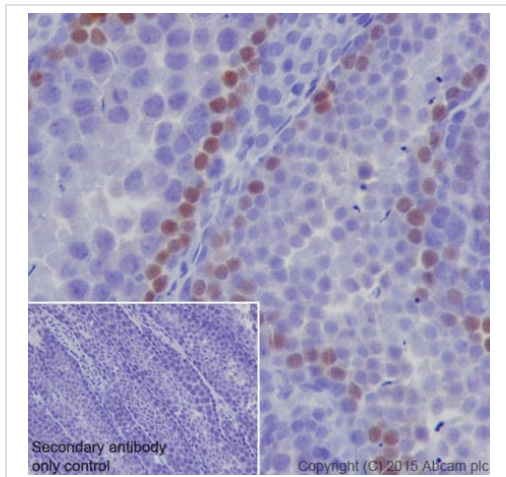
Lanes 1 - 4: Merged signal (red and green). Green - ab195268 observed at 125 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

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.



Immunocytochemistry/ Immunofluorescence - Anti-Bub1 antibody [EPR18947] (ab195268)

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)

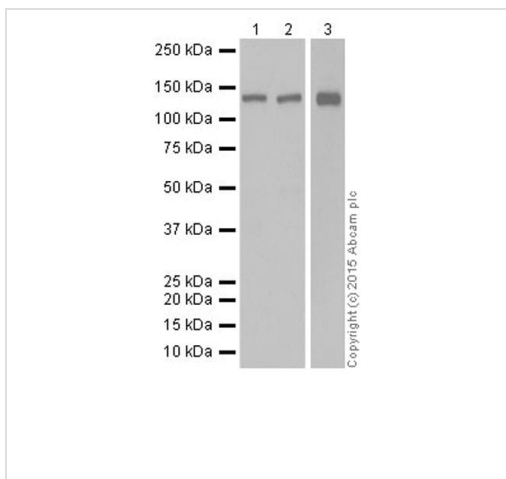
Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling Bub1 with ab195268 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution.

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

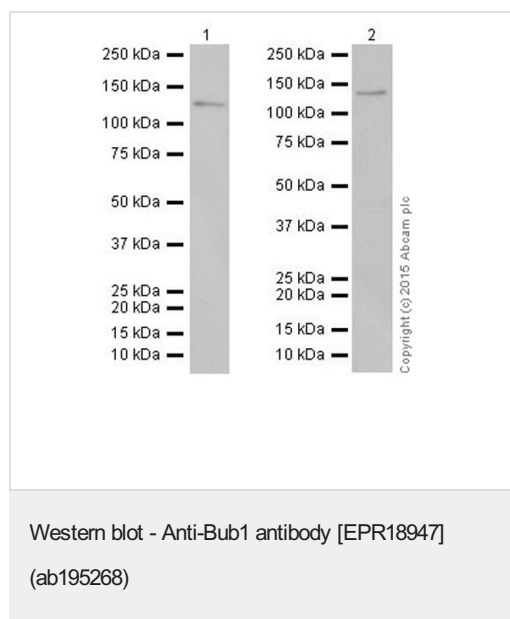
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

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.



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

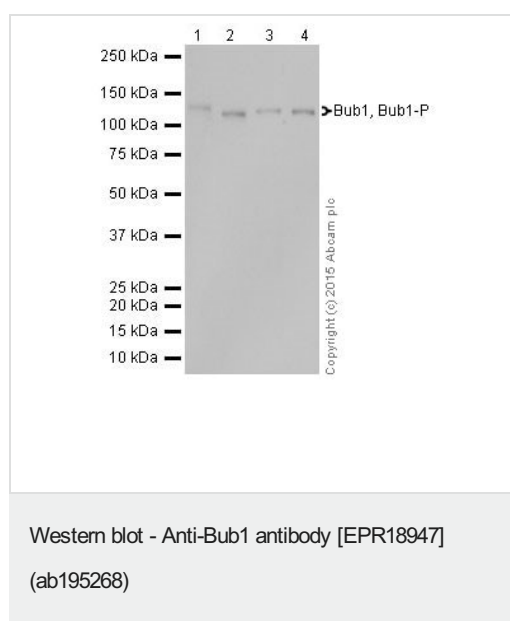
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 122 kDa

Observed band size: 122 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



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

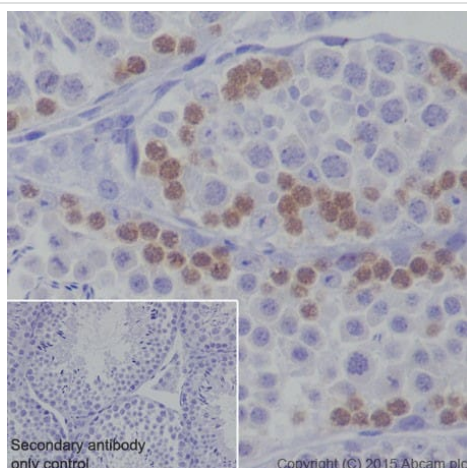
Observed band size: 122 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

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.

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

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Bub1 antibody [EPR18947] (ab195268)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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