abcam

ab54893

Anti-Bub1 antibody [2F9]

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human BUB1 aa 1-150. The exact immunogen used to generate this antibody is proprietary information. Database link O43683
Clone number	2F9
Purity	Tissue culture supernatant
Light chain type	kappa

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target data

See full target information BUB1

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. Required for centromeric enrichment of AUKRB in prometaphase. Plays an important role in defining SGO1 localization and thereby affects sister chromatid cohesion. Promotes the centromeric localization of TOP2A (PubMed:35044816). 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.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

This product was changed from ascites to tissue culture supernatant on 25/02/19. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Bub1 protein often referred to as 'Bub1' plays a mechanical role in cell cycle regulation especially during mitosis. It is known by other names such as BUB1 mitotic checkpoint serine/threonine kinase. Bub1 has a molecular mass of approximately 120 kDa. This protein is expressed in various tissues with higher levels in rapidly dividing cells. The location of its action is mainly the nucleus where it associates with kinetochores during cell division functioning in the spindle assembly checkpoint.
Biological function summary	Bub1 protein acts as a serine/threonine-protein kinase that is essential for proper chromosome segregation. It forms part of the spindle checkpoint complex along with other proteins such as BubR1 and Mad2. This complex ensures that chromosomes are correctly attached to the spindle microtubules before anaphase begins. Bub1's kinase activity contributes to the prevention of premature anaphase onset thereby helping maintain genomic stability.
Pathways	Bub1 is significantly involved in the mitotic checkpoint pathway and the cell cycle control pathway. Within these pathways Bub1 interacts closely with proteins like CDC20 and APC/C which are integral parts of the anaphase-promoting complex. Bub1 modifies the activity of these proteins to delay anaphase onset until all chromosomes are properly aligned ensuring the fidelity of cell division.
Associated diseases and disorders	Bub1 protein shows a connection to various cancer types notably breast cancer and colorectal cancer. Defects or overexpression in Bub1 can disrupt the spindle assembly checkpoint leading to chromosome missegregation and aneuploidy hallmarks of cancerous cells. Bub1 is also linked to other spindle checkpoint proteins such as Mad1 and Bub3. Malfunction in any of these proteins including Bub1 can contribute to tumorigenesis due to impaired cell cycle control.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

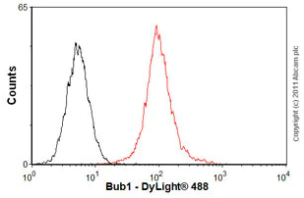
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

6 product images



Flow Cytometry - Anti-Bub1 antibody [2F9] (ab54893)

Bub1 Flow Cytometry staining using mouse Anti-Bub1 antibody

Overlay histogram showing HeLa cells stained with ab54893 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab54893, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.
This image was generated using the ascites version of the product.

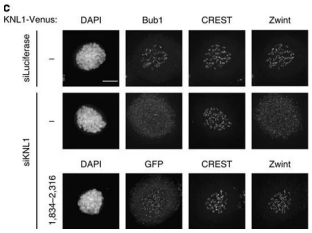


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Immunocytochemistry/ Immunofluorescence - Anti-Bub1 antibody [2F9] (ab54893)

Bub1 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Bub1 antibody

Immunocytochemistry-immunofluorescence using Anti-Bub1 antibody [2F9], ab54893. Publication image from Nilsson, J. et al., 2015, Nat Commun, 26031201. Legend direct from paper.

ZW10 kinetochore localization is not supported by a KNL1 fragment containing the Zwint binding domain.(a) Outline of the synchronization protocol used in this study. Cells were fixed 2 h after addition of nocodazole. (b) Schematic of human KNL1 with functional regions indicated. The region encoded by KNL1 1,834–2,316 and KNL1 1,834–2,316+4XMELT is depicted below. (c) HeLa cells were treated with a control RNAi oligo (luciferase) or an RNAi oligo targeting KNL1 for 48 h and arrested in mitosis using nocodazole for 2 h. In one condition, cells were co-transfected with a plasmid encoding KNL1 1,834–2,316-Venus as indicated. Cells were fixed and stained with DAPI, CREST (centromere marker) and Zwint. We used Bub1 as a KNL1 depletion marker because both our KNL1 and Zwint antibodies are rabbit polyclonal. Bub1 solely depends on KNL1 for its localization and its kinetochore levels follow KNL1. The expression of KNL1 1,834–2,316 Venus was detected by staining cells with a GFP antibody. (d,e) The kinetochore levels of Bub1 and Zwint were determined in the indicated conditions and normalized to CREST. (f,g) As in c–e, but cells were stained with a ZW10 antibody and the kinetochore levels of ZW10 were measured and normalized to CREST in the indicated conditions. At least 160 single kinetochores from eight different cells were measured in all the conditions and the mean with standard error of mean is indicated. Scale bar, 5 µm.

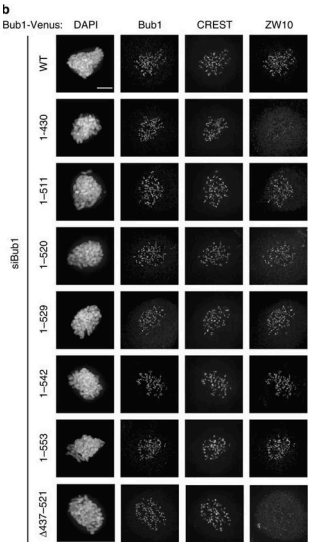


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Bub1 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Bub1 antibody

Immunocytochemistry-immunofluorescence using Anti-Bub1 antibody [2F9], ab54893. Publication image from Nilsson, J. et al., 2015, Nat Commun, 26031201. Legend direct from paper.

A region encompassing CD1 in Bub1 is required for RZZ localization.(a) Schematic of Bub1 primary structure with motifs indicated and below a schematic of the different truncation constructs used in this study. All constructs contain the Bub3 binding site ensuring kinetochore targeting. (b) HeLa cells were depleted of endogenous Bub1 using RNAi and the indicated Bub1 constructs were co-transfected for 48 h and cells were arrested with nocodazole for 2 h. Cells were fixed and stained for Bub1, CREST and ZW10. (c,d) The Bub1 (c) or ZW10 (d) kinetochores levels was measured and normalized to CREST signals. At least 160 single kinetochores were analysed from eight different cells and the mean with standard error of mean is indicated. A t-test was used to compare the values in d. (NS, not significant (P>0.05), *P≤0.05, **P≤0.01, ****P≤0.0001). Scale bar, 5 µm.

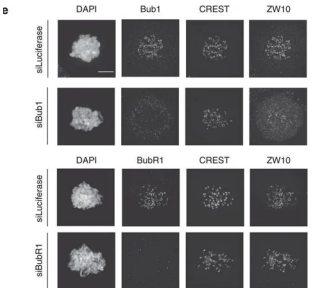


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Immunocytochemistry-immunofluorescence using Anti-Bub1 antibody [2F9], ab54893. Publication image from Nilsson, J. et al., 2015, Nat Commun, 26031201. Legend direct from paper.

Bub1 is required for RZZ localization to kinetochores.(a) HeLa cells were depleted of endogenous KNL1 using RNAi and the indicated KNL1 constructs were co-transfected for 48 h and cells were arrested with nocodazole for 2 h. Cells were fixed and stained for GFP (KNL1 constructs), CREST and ZW10. (b) The ratios of ZW10/CREST and GFP/CREST signal were calculated. (c) As in a with the ZW10/CREST ratio shown in d. The GFP/CREST values for the three KNL1 constructs were: KNL1 1,834–2,316: 1.00; KNL1 1,834–2,316+4XMELT: 1.11; KNL1 1,834–2,316 4XMELA: 0.85. (e) HeLa cells were treated with luciferase, BubR1 or Bub1 RNAi for 48 h and cells were arrested with nocodazole for 2 h. Cells were fixed and stained for Bub1, BubR1, CREST and ZW10 as indicated. (f) The kinetochore levels of ZW10 were measured and normalized to CREST and at least 160 single kinetochores from eight different cells was measured in all the conditions and the mean with standard error of mean is indicated. Scale bar, 5 µm.

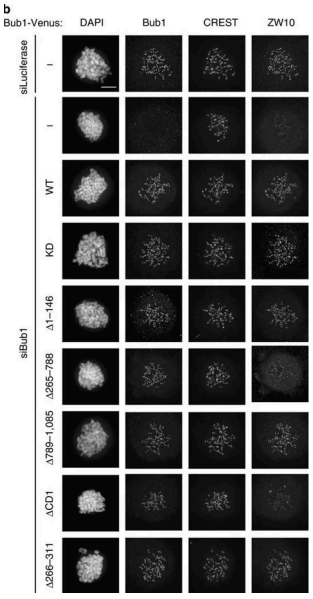


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Immunocytochemistry-immunofluorescence using Anti-Bub1 antibody [2F9], ab54893. Publication image from Nilsson, J. et al., 2015, Nat Commun, 26031201. Legend direct from paper.

The central region of Bub1 is required for RZZ recruitment.(a) Schematic of Bub1 primary structure with motifs indicated and below a schematic of different deletion and mutation constructs used in this study. All Bub1 constructs contain the Bub3 binding site ensuring kinetochore targeting. (b) HeLa cells were depleted of endogenous Bub1 using RNAi and the indicated Bub1 constructs were co-transfected for 48 h and cells were arrested with nocodazole for 2 h. Cells were fixed and stained for Bub1, CREST and ZW10. The expression of the exogenous Bub1 construct could be detected by the Bub1 antibody that recognize an epitope in the N terminus of Bub1 except for Bub1 δ1–146. *To detect Bub1 δ1–146 expression cells were stained with a GFP antibody. To determine if Bub1 δ1–146 could recruit ZW10 to kinetochores it was compared with cells expressing wild-type Bub1-Venus at a GFP signal level at which wild-type Bub1 could fully rescue ZW10 localization. The Venus levels were normalized to wild-type Bub1-Venus levels (set to 1) and incorporated into c. (c,d) The Bub1 (c) or ZW10 (d) intensity at kinetochores was measured and normalized to CREST. At least 160 single kinetochores were analysed from eight different cells and the mean with standard error of mean is indicated. Scale bar, 5 µm.

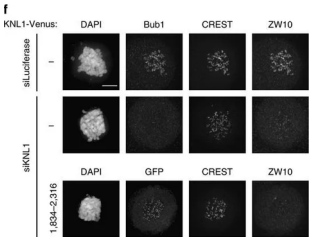


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