Datasheet for ABIN968647

anti-BUB3 antibody (AA 4-16)

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Overview

Quantity:	50 µg
Target:	BUB3
Binding Specificity:	AA 4-16
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This BUB3 antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Immunogen:	Human Bub3 aa. 4-16
Clone:	31-Bub3
Isotype:	IgG1
Cross-Reactivity:	Mouse (Murine), Rat (Rattus)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance. 3. Triton is a trademark of the Dow Chemical Company. 4. Source of all serum proteins is from USDA inspected abattoirs located in the United States. 5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide

Product Details

	compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing. 6. Please refer to us for technical protocols.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	BUB3
Alternative Name:	Bub3 (BUB3 Products)
Background:	Accurate chromosome segregation requires that all pairs of sister chromatids become appropriately attached to mitotic spindles before the onset of anaphase. Cell cycle checkpoints monitor kinetochore-microtubule interactions, so that cell cycle progression can be delayed until proper chromosome attachments are formed. In yeast, Bub1-3 genes are required for proper mitotic delay in response to unattached kinetochores. In mammals, the homologues to yeast Bub1 and Bub3 form a complex that binds kinetochores and has protein kinase activity. Bub3 contains four WD repeats, three in the N-terminus and one in the C-terminus, and a central Bub1-binding domain. During prophase and prometaphase, Bub3 localizes to the kinetochore before attachment to microtubules. In addition, taxol-induced formation of lagging chromosomes due to a delay of cell cycle progression increases the level of Bub3 co-localized with kinetochores, while correctly aligned chromosomes found in metaphase do not exhibit this co-localization. Thus, Bub3, in association with Bub1, may be important for sensing kinetochore attachment to microtubules during the prometaphase to metaphase transition.
Molecular Weight:	40 kDa
Pathways:	Maintenance of Protein Location

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes

Application Details

	at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT. 4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT. 7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT. 9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C.

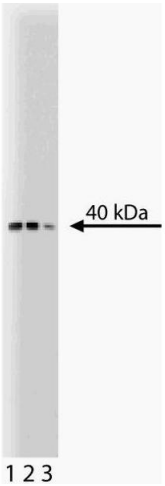
Publications

Product cited in:	Martinez-Exposito, Kaplan, Copeland, Sorger: "Retention of the BUB3 checkpoint protein on lagging chromosomes." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 96, Issue 15, pp. 8493-8, (1999) (PubMed). Taylor, Ha, McKeon: "The human homologue of Bub3 is required for kinetochore localization of

Publications

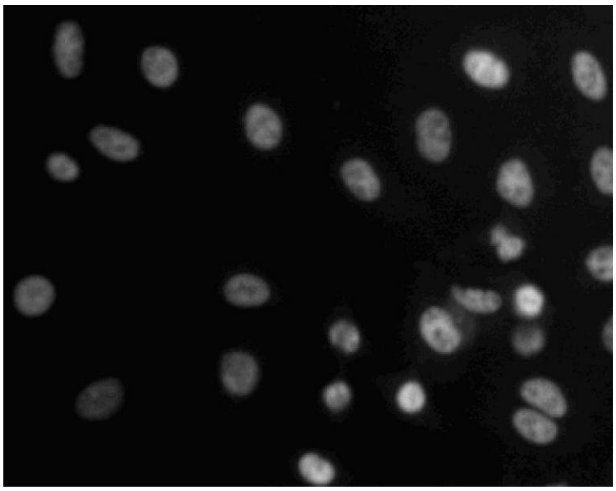
Bub1 and a Mad3/Bub1-related protein kinase." in: **The Journal of cell biology**, Vol. 142, Issue 1 , pp. 1-11, (1998) (PubMed).

Images



Western Blotting

Image 1. Western blot analysis of Bub3 on a SW13 lysate. Lane 1: 1:500, lane 2: 1:1000, lane 3: 1:2000 dilution of the Mouse Anti-Bub3 antibody.



Immunofluorescence

Image 2. Immunofluorescent staining of A549 (ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the Mouse Anti-Bub3 antibody. The second step reagent was FITC goat anti mouse Ig. The image was taken on a BD Pathway™ 855 bioimagin system using a 20x objective. This antibody also stains HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells and can be used with either fix/perm protocol.