Technical Data Sheet

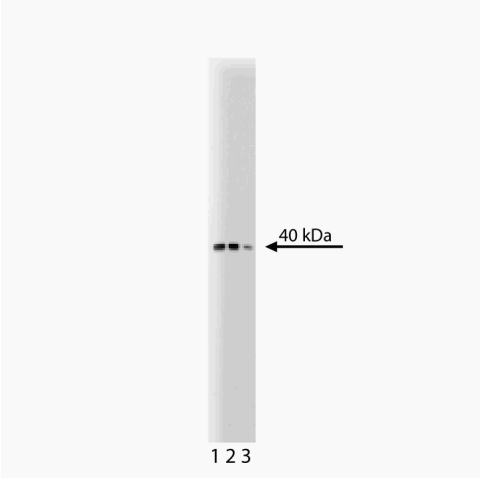
Purified Mouse Anti-Bub3

Product Information

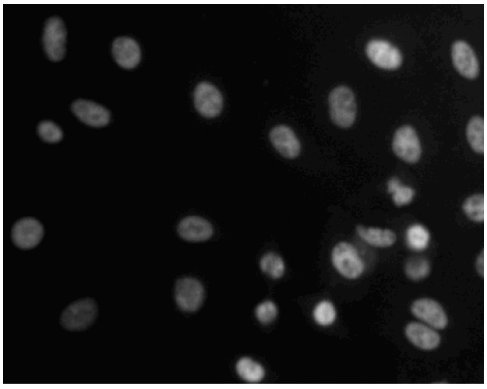
Material Number:	**611730**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	31/Bub3
Immunogen:	Human Bub3 aa. 4-16
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat
Target MW:	40 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

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.



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.*



Immunofluorescent staining of A549 (ATCC CCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) 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 (Cat. No. 554001). 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 (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

BD Biosciences

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl 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 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl 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 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl 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 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

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 compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Martinez-Exposito MJ, Kaplan KB, Copeland J, Sorger PK. Retention of the BUB3 checkpoint protein on lagging chromosomes. *Proc Natl Acad Sci U S A*. 1999; 96(15):8493-8498. (Biology)

Taylor SS, Ha E, McKeon F. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. *J Cell Biol*. 1998; 142(1):1-11. (Biology)