BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

Purified Mouse Anti-β-Tubulin

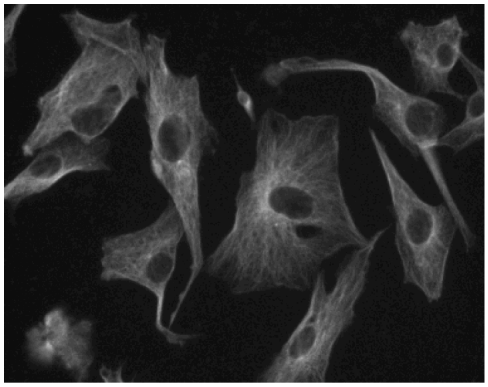
Product Information

Material Number:	**556321**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	5H1
Isotype:	Mouse IgM, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Cow
Target MW:	50 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Tubulin is a highly conserved protein with a molecular weight of ~50 kD. The self-assembly of tubulin leads to microtubules, hollow cylinders that are one of the major components of the eukaryotic cytoskeleton. Microtubules play key roles in chromosome segregation in mitosis, intracellular transport, ciliary and flagellar bending, and structural support of the cytoskeleton. There are two main classes of tubulin isoforms, α- and β-tubulin, which are usually products of separate genes. Microtubules are made from protofilaments, strings of alternating α- and β-tubulin spaced 4 nm apart and pointing in the same direction. Tubulin can be posttranslationally modified in several ways, including phosphorylation, acetylation, glutamylation, and detyrosination. For example, microtubules that turn over slowly tend to be acetylated and detyrosinated.

The 5H1 monoclonal antibody reacts with β-tubulin. It does not cross-react with α-tubulin.



Immunofluorescent staining of U-87 MG (ATCC HTB-14) 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 (see Recommended Assay Procedure) and the anti-Tubulin antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stained A-431 (ATCC CRL-1555), HeLa (ATCC CCL-2), A549 (ATCC CCL-185) 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 4°C.

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

Application Notes

Application

Western blot	Routinely Tested
Immunohistochemistry	Tested During Development
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.

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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
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)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 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. 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.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Brown KD, Wood KW, Cleveland DW. The kinesin-like protein CENP-E is kinetochore-associated throughout poleward chromosome segregation during anaphase-A. *J Cell Sci.* 1996; 109:961-969. (Biology)

Cho J-H, Johnson GVW. Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3β (GSK3β) plays a critical role in regulating tau's ability to bind and stabilize microtubules. *J Neurochem.* 2004; 88:349-358. (Biology)

Helfand BT, Mikami A, Vallee RB, Goldman RD. A requirement for cytoplasmic dynein and dynactin in intermediate filament network assembly and organization. *J Cell Biol.* 2002; 157(5):795-806. (Biology)

Prahlad V, Yoon M, Moir RD, Vale RD, Goldman RD. Rapid movements of vimentin on microtubule tracks: kinesin-dependent assembly of intermediate filament networks. *J Cell Biol.* 1998; 143(1):159-170. (Biology)

Wang Y, Loomis PA, Zinkoswski RP, Binder LI. A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau. *J Cell Biol.* 1993; 121(2):257-267. (Biology)