BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

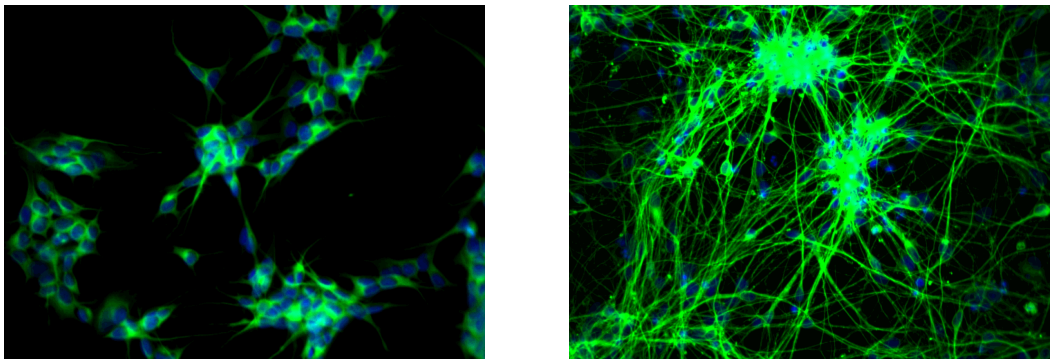
Alexa Fluor® 488 Mouse anti-β-Tubulin, Class III

Product Information

Material Number:	**560338**
Alternate Name:	tubulin, beta 3; MC1R; TUBB3; TUBB4; tubulin, beta-4
Size:	100 tests
Vol. per Test:	5 µl
Clone:	TUJ1
Immunogen:	Rat brain microtubules
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human. Reported: Rat.
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Microtubules are formed by the self assembly of tubulin and are one of the major components of the eukaryotic cytoskeleton. The two main tubulin isoforms, α - and ß-tubulin, are usually products of separate genes. The ß-tubulin family includes six expressed genes that produce the polypeptide isoforms known as Classes I through VI, each of which have a distinct pattern of expression. Class III ß-tubulin is found in neurons and mammalian testis cells and is widely used as a neuronal marker in developmental neurobiology, neoplasia, and stem cell research. Class III ß-tubulin expression in neuronal and neuroblastic tumors is differentiation dependent, and its expression in certain non-neuronal neoplasms has been associated with poor prognosis and/or resistance to chemotherapy.



Immunofluorescent staining of human cell lines. LEFT image: *SH-SY5Y neuroblastoma cells (ATCC, Cat. No. CRL-2266) were seeded in a BD Falcon™ 96-well imaging plate (Cat. No. 353219) at ~10,000 cells per well in culture medium and incubated overnight.* ***RIGHT image:*** *H9 embryonic stem cells (WiCell, Madison, WI) were differentiated into neural stem cells (NCS). The NCS were seeded on polyornathine and laminin-coated BD Falcon™ 96-well Imaging Plates (Cat. No. 353219) and allowed to differentiate for an additional 2 weeks. The cells were fixed, permeabilized with cold methanol, and stained with Alexa Fluor® 488 Mouse anti-β-Tubulin, Class III (pseudo colored green), and the nuclei were counterstained with Hoechst 33342 (pseudo colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 High-Content Bioimager System using a 20x objective and merged using the BD AttoVison™ software. This antibody did not stain HeLa epithelial adenocarcinoma cells (ATCC CCL-2). This antibody also worked with both the Triton X100 and Saponin fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

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

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Bioimaging	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Recommended Assay Procedure:

For more information, please refer to: http://www.bdbiosciences.com/pharmingen/protocols/Bioimaging_Certified.shtml

Recommended Protocol for Bioimaging:

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
    a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
    b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
    c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	488/10	515 LP	Fura/FITC
BD Pathway 435	482/35	536/40	FF506

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Triton is a trademark of the Dow Chemical Company.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Geisert EE Jr, Frankfurter A. The neuronal response to injury as visualized by immunostaining of class III beta-tubulin in the rat. *Neurosci Lett.* 1989; 102(2-3):137-141. (Clone-specific: Immunohistochemistry)

Katsetos CD, Del Valle L, Geddes JF et al. Aberrant localization of the neuronal class III beta-tubulin in astrocytomas. *Arch Pathol Lab Med.* 2001; 125(5):613-624. (Clone-specific: Immunocytochemistry (cytospins))

Mozzetti S, Ferlini C, Concolino P et al. Class III beta-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. *Clin Cancer Res.* 2005; 11(1):298-305. (Clone-specific: Immunohistochemistry)