Technical Data Sheet

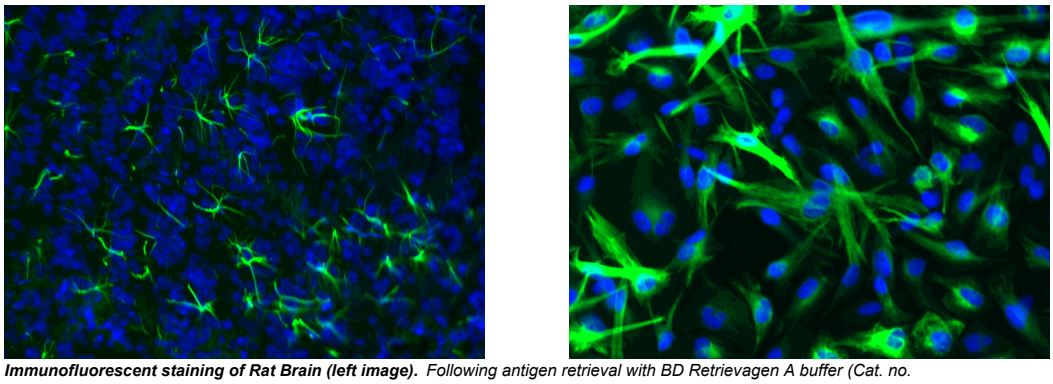
Alexa Fluor® 488 Mouse anti-GFAP

Product Information

Material Number:	**560297**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	1B4
Immunogen:	Cow spinal cord homogenate
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human Reported by Western Blot (Cat. No. 556328): Rat, Mouse, Cow, Sheep, Dog, Pig, Rabbit, Guinea Pig, Chicken
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

GFAP (**G**lial **F**ibrillary **A**cidic **P**rotein) is the major protein of glial filaments in differentiated astrocytes. BD Biosciences offers a panel of monoclonal antibodies (4A11, 1B4, 2E1) that specifically recognize GFAP. They do not cross-react with other intermediate filaments such as vimentin, neurofilament proteins, desmin, keratin, neurotubules or microfilaments.



Immunofluorescent staining of Rat Brain (left image). *Following antigen retrieval with BD Retrievagen A buffer (Cat. no. 550524), the formalin-fixed paraffin-embedded sections were stained with Alexa Fluor® 488 Mouse anti-GFAP (pseudo colored green) and counterstained with Hoechst 33342 (pseudo colored blue). The images were captured on a BD Pathway™ 435 High-Content Bioimager System with a 20x objective and merged using BD Attovision™ software.*
Immunofluorescent staining of human grade III glioblastoma and astrocytoma cell line (right image). *U-373 (ATCC HTB-17; discontinued, investigators may refer to: http://www.atcc.org/MisidentifiedCellLines/tabid/683/Default.aspx) were seeded in a 96-well imaging plate (Cat. No. 353219) at ~15,000 cells per well. After overnight incubation, the cells were fixed, permeabilized with cold methanol, and stained with Alexa Fluor® 488 Mouse anti-GFAP (pseudo colored green) according to the Recommended Assay Procedure. Cell nuclei were counterstained with Hoechst 33342 (pseudo colored blue). The images were captured on a BD Pathway™ 435 High-Content Bioimager System using a 20X objective and merged using BD AttoVision™ software. It also worked with the Saponin and the Triton X-100 Perm/Wash protocols (see Recommended Assay Procedure; Bioimaging protocol link).*

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

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.

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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)
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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.
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

McLendon RE, Bigner DD. Immunohistochemistry of the glial fibrillary acidic protein: basic and applied considerations. *Brain Pathol.* 1994; 4(3):221-228. (Biology: Immunohistochemistry)

McLendon RE, Burger PC, Pegram CN, Eng LF, Bigner DD. The immunohistochemical application of three anti-GFAP monoclonal antibodies to formalin-fixed, paraffin-embedded, normal and neoplastic brain tissues. *J Neuropathol Exp Neurol.* 1986; 45(6):692-703. (Biology: Immunohistochemistry, Western blot)

Pegram CN, Eng LF, Wikstrand CJ, McComb RD, Lee YL, Bigner DD. Monoclonal antibodies reactive with epitopes restricted to glial fibrillary acidic proteins of several species. *Neurochem Pathol.* 1985; 3(2):119-138. (Biology)

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