

## Technical Data Sheet

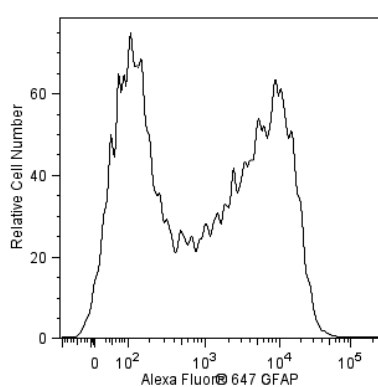
## Alexa Fluor® 647 Mouse anti-GFAP

## Product Information

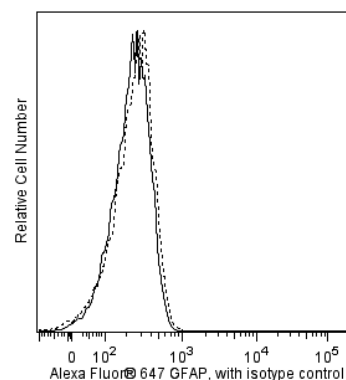
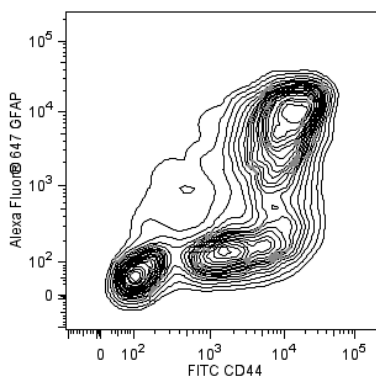
<b>Material Number:</b>	<b>561470</b>
<b>Alternate Name:</b>	Glial Fibrillary Acidic Protein, FLJ45472
<b>Size:</b>	50 tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	1B4
<b>Immunogen:</b>	Cow spinal cord homogenate
<b>Isotype:</b>	Mouse IgG2b
<b>Reactivity:</b>	QC Testing: Human Reported by Western Blot (Cat. No. 556328): Rat, Mouse, Cow, Sheep, Dog, Pig, Rabbit, Guinea Pig, Chicken
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

## Description

GFAP (Glial Fibrillary Acidic Protein) 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.



**Analysis of GFAP in differentiated human Neural Stem Cells (NSC).** NSC derived from H9 cells (WiCell, Madison, WI) were differentiated in NSC differentiation medium [containing N2 and B-27 supplements (Life Technologies), recombinant human BDNF and GDNF (Peprotech), dibutyl cyclic AMP (Sigma)] for 11 days followed by AGM™ Astrocyte Growth Medium (Lonza) for 16 days. The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 20 minutes at room temperature, permeabilized with BD™ Phosflow Perm/Wash Buffer I (Cat. No. 557885), and then stained with Alexa Fluor® 647 Mouse anti-GFAP (left panel) and co-stained with FITC mouse anti-CD44 (Cat. No. 555478) as shown in the right panel. This antibody conjugate also works with BD™ Phosflow Perm Buffer III. Flow cytometry was performed on a BD LSR™ II flow cytometer.



**Analysis of GFAP in Neural Stem cells (NSC).** NSCs were isolated by sorting from Embryoid bodies and were grown for 8 passages post sort, fixed (BD Cytofix™ buffer, Cat. No. 554655) for 20 minutes at room temperature, permeabilized with BD™ Phosflow Perm Buffer I (Cat. No. 557885), and then stained with either Alexa Fluor® 647 Mouse anti-GFAP (solid line) or Alexa Fluor® 647 Mouse IgG2b, κ Isotype Control (Cat. No. 558713, dashed line).

## Preparation and Storage

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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

## Application Notes

## Application

Intracellular staining (flow cytometry)

Routinely Tested

## BD Biosciences

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**Recommended Assay Procedure:**

The formulation of this antibody conjugate has been optimized for flow cytometry. We recommend Cat. No. 560298 for Bioimaging.

**Suggested Companion Products**

<b>Catalog Number</b>	<b>Name</b>	<b>Size</b>	<b>Clone</b>
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558713	Alexa Fluor® 647 Mouse IgG2b, κ Isotype Control	50 tests	27-35

**Product Notices**

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-μl experimental sample (a test).
2. Please refer to [www.bdbiosciences.com/pharmlingen/protocols](http://www.bdbiosciences.com/pharmlingen/protocols) for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. 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.
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. 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)

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)

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)