Technical Data Sheet

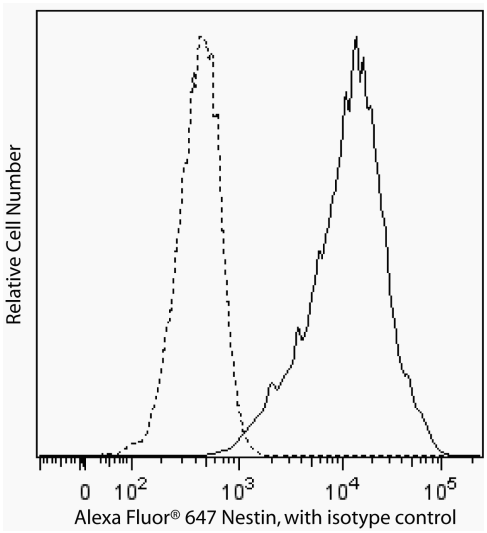
Alexa Fluor® 647 Mouse Anti-Nestin

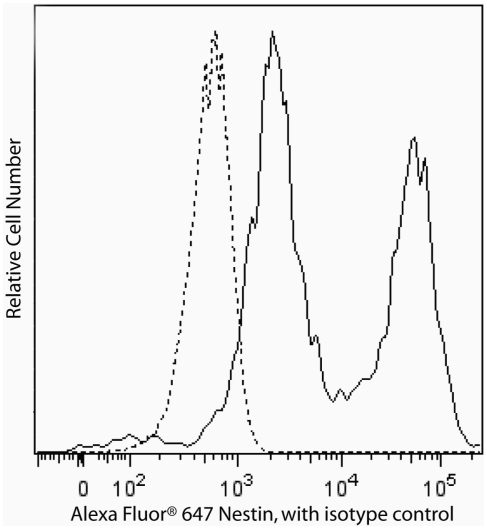
Product Information

Material Number:	**560393**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	25/NESTIN
Immunogen:	Rat Nestin aa. 402-604 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Rat Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The cytoskeleton consists primarily of core structural proteins that include microfilaments, microtubules, and intermediate filaments (IFs). IFs contain more than 50 distinct proteins that are organized into six different subtypes: Type I/II keratins expressed in epithelia, type III vimentin/desmin, type IV neurofilament proteins, type V nuclear lamins, and type VI nestin expressed primarily in embryonic cells. Nestin has a conserved core region (amino acids 7 to 314), which contains an α helical domain that is involved in coiled-coil assembly of IFs. The C-terminal region of nestin is similar to type IV IFs, since it contains highly charged amino acids, many glutamate residues, and an 11 amino acid repeat motif. Nestin is expressed in the cerebrum during embryonic development, in the cerebellum during early postnatal development, and in dermatomal cells and myoblasts during myogenesis. In vitro, nestin forms homodimers and homotetramers, but not IFs, and can co-assemble with type III vimentin and type IV internexin proteins. Thus, nestin is a core IF protein that is essential for proper cytoskeletal formation during neurogenesis and myogenesis.





LEFT Image: Analysis of Alexa Fluor® 647 Mouse Anti-Nestin on Neural Stem cells (NSCs). *H9-derived NSCs were isolated by sorting from Embryoid bodies and were grown for 2 passages post sort, fixed (BD Cytofix™ Fixation 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-Nestin (solid line) or Alexa Fluor® 647 Mouse IgG1, κ Isotype Control (Cat. No. 557732, dashed line). Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.*

RIGHT Image: Analysis of Alexa Fluor® 647 Mouse Anti-Nestin on Neurons. *H9-derived NSC were differentiated into neurons for 12 days. The cells were fixed (BD Cytofix™ Fixation 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 Alex Fluor® 647 Mouse anti-Nestin (solid line) or Alexa Fluor® 647 Mouse IgG1, κ Isotype Control (Cat.No. 557732, dashed line). The two peaks reveal differentiated neurons (low Nestin staining) and undifferentiated NSC (high Nestin staining). This antibody also works in BD Phosflow™ Perm Buffers II and III. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.*

Preparation and Storage

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

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.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
557885	Perm/Wash Buffer I	125 ml	(none)
557732	Alexa Fluor® 647 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. 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.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Kachinsky AM, Dominov JA, Miller JB. Myogenesis and the intermediate filament protein, nestin. *Dev Biol.* 1994; 165(1):216-228. (Biology)

Kernie SG, Erwin TM, Parada LF. Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. *J Neurosci Res.* 2001; 66(3):317-326. (Clone-specific: Immunofluorescence)

Lendahl U, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. *Cell.* 1990; 60(4):585-595. (Biology)

Steinert PM, Chou YH, Prahlad V, et al. A high molecular weight intermediate filament-associated protein in BHK-21 cells is nestin, a type VI intermediate filament protein. Limited co-assembly in vitro to form heteropolymers with type III vimentin and type IV alpha-internexin. *J Biol Chem.* 1999; 274(14):9881-9890. (Biology)