Technical Data Sheet

V450 Mouse anti-Nestin

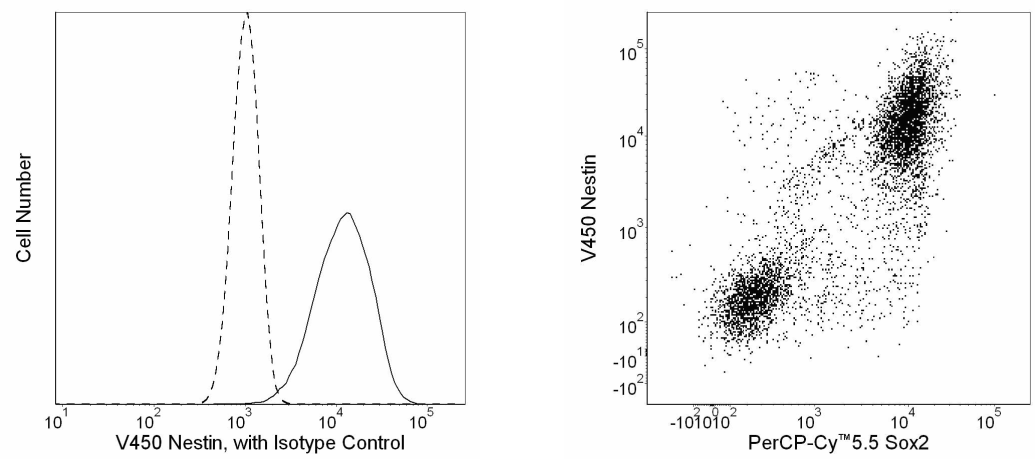
Product Information

Material Number:	**561551**
Size:	50 tests
Vol. per Test:	5 µl
Clone:	25/NESTIN
Immunogen:	Rat Nestin aa. 402-604 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Rat Reported: Human
Storage Buffer:	Aqueous buffered solution containing 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.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the **V**iolet laser Ex max of 406 nm and has an Em Max at **450** nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Analysis of Nestin staining on H9 derived Neural Stem Cells (NSC, left) and Neurons (right). *NSC and neurons were derived from H9 human embryonic stem cells (WiCell, Madison, WI). The NSC and neurons were harvested, fixed in BD Cytofix™ buffer (Cat. No. 554655), and permeabilized with BD™ Phosflow Perm/Wash buffer III (Cat. No. 558050). The left panel shows NSC that were stained with matching concentrations of either BD Horizon™ V450 Mouse IgG1, κ isotype control (dashed line, Cat. No.560373) or V450 Mouse anti-Nestin monoclonal antibody (solid line). The right panel shows neurons that were stained with V450 Mouse anti-Nestin and PerCP-Cy™5.5 Mouse anti-Sox2 (Cat. No. 561506) monoclonal antibodies. The histograms were derived from gated events based on the light scattering characteristics of NSC and neurons, respectively. Flow cytometry was performed on a BD LSR™ II flow cytometry system. The neurons were derived from a sorted population of H9-derived NSC that were then differentiated for 4 weeks in NSC differentiation medium [containing N2/B27 supplement (Life Technologies), BDNF, GDNF (Peprotech), and dibutryl cyclic-AMP (Sigma)]. The double-positive population consists of NSC and glial cells, while the double-negative population consists primarily of neurons.*

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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 with BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
560373	V450 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554656	Stain Buffer (FBS)	500 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. Cy is a trademark of Amersham Biosciences Limited.
8. 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.

References

Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc Natl Acad Sci U S A.* 2002; 99(18):11946-11950. (Clone-specific: Immunofluorescence)

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)

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Wu D, Tadano M, Edamatsu H, et al. Neuronal lineage-specific induction of phospholipase Cepsilon expression in the developing mouse brain. *Eur J Neurosci.* 2003; 17(8):1571-1580. (Clone-specific: Immunofluorescence, Immunohistochemistry)

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