Technical Data Sheet

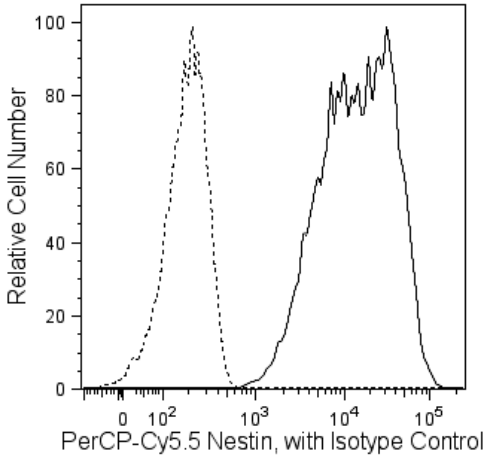
PerCP-Cy™5.5 Mouse anti-Nestin

Product Information

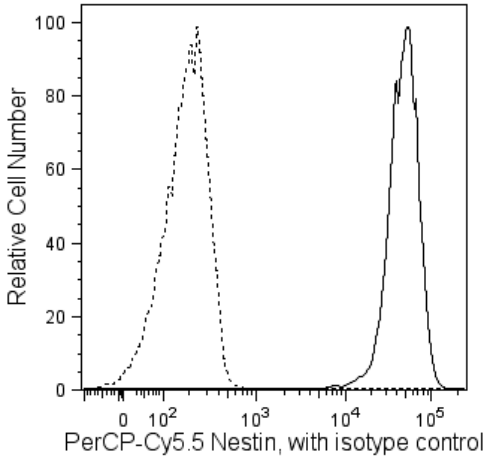
Material Number:	**561231**
Size:	50 tests
Vol. per Test:	5 µl
Clone:	25/NESTIN
Immunogen:	Rat Nestin aa. 402-604 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Rat Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

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.



Analysis of Nestin expression in rat glioma. *C6 cells (ATCC, Cat. No. CCL-107) were 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 PerCP-Cy™5.5 Mouse anti-Nestin (solid line) or PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control (Clone MOPC-21, Cat. No. 550795, dashed line). Flow cytometry was performed on a BD LSR™ II flow cytometry system. This antibody conjugate is also compatible with BD Phosflow™ Perm Buffers II and III.*



Analysis of Nestin expression in human Neural Stem Cells (NSC). *NSC were derived from H9 human embryonic stem cells (WiCell, Wisconsin) and grown for 8 passages, 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 PerCP-Cy™5.5 Mouse anti-Nestin (solid line) or PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control (Clone MOPC-21, Cat. No. 550795, dashed line). Flow cytometry was performed on a BD LSR™ II flow cytometry system. This antibody conjugate is also compatible with BD Phosflow™ Perm Buffers II and III.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human and rat cell lines using BD Cytofix™ Fixation Buffer and the BD Phosflow™ Permeabilization Buffers: Perm/Wash Buffer I, Perm Buffer II, or Perm Buffer III (see Suggested Companion Products).

Suggested Companion Products

Catalog Number	Name	Size	Clone
550795	PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. 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.
10. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
11. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.

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)

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)

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)