

Reagents Provided

NorthernLights™ 557 (NL557)-conjugated mouse monoclonal anti-human Nestin: Supplied as 50 μ g of antibody in 0.5 mL PBS containing 0.1% sodium azide.

Clone #: 196908

Isotype: mouse IgG₁

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to visualize the expression of Nestin by fluorescence microscopy.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with NS0 cells transfected with amino acids 618 - 1618 of human Nestin. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to fluorochrome NL557. The spectral characteristics of NL557 are provided, along with those of Rhodamine Red™-X (RRX) and Cy™3 for comparison.

Fluorochrome	Absorption Maximum (nm)	Emission Maximum (nm)
NL557	557	574
RRX	570	590
Cy™3	548	562

Background Information

Nestin is a class VI intermediate filament protein^{1,2} that is expressed in stem cells of the central nervous system (CNS)³ but not in mature CNS cells.⁴ Nestin expression is used extensively as a marker for CNS stem cells in the developing nervous system and *in vitro* cultured cells.⁵⁻¹⁰ Its transient expression is a critical step in the neural differentiation pathway.² Nestin is also expressed in non-neural stem cell populations, such as pancreatic islet progenitors¹¹⁻¹³ and hematopoietic progenitors.¹⁴ This antibody was selected for its ability to detect Nestin on human neural progenitors and A172 cells by immunocytochemistry and intracellular flow cytometry. This antibody does not react with Nestin in mouse or rat neural stem cells by immunocytochemistry.

References

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Immunocytochemistry Validation

This antibody has been tested for immunocytochemistry using NTERA-2 cells. Cells were fixed in PBS containing 4% paraformaldehyde, and blocked with PBS containing 10% normal donkey serum, 0.1% Triton® X-100, and 1% BSA. After blocking, cells were incubated with NL557-conjugated antibody at a final concentration of 10 μ g/mL (1:10 dilution) in blocking buffer for 3 hours at room temperature, or overnight at 4° C, in the dark. Between each step, cells were washed with PBS containing BSA. If a staining volume of 250 μ L is used, this kit can be used for 20 tests; 100 tests can be done in a staining volume of 50 μ L.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.