

## Reagents Provided

**NorthernLights™ 557 (NL557)-conjugated mouse monoclonal anti-rat Nestin:** Supplied as a 10X solution of antibody in 0.5 mL PBS containing 0.1% sodium azide.

**Clone #:** 307501

**Isotype:** mouse IgG<sub>2a</sub>

## Storage

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

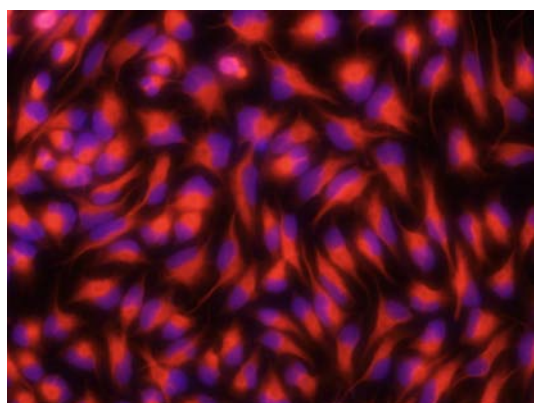
## Intended Use

Designed to visualize the expression of rat 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 purified, *E. coli*-derived, recombinant rat Nestin (rrNestin; aa 544 - 776). 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
Cy3	548	562



**Rat Nestin-NL557**

Undifferentiated rat cortical stem cells were stained with NL557-conjugated anti-rat Nestin (Catalog # NL2736R, red) and counterstained with DAPI (blue).

## Background Information

Nestin is a class VI intermediate filament protein<sup>1,2</sup> that is expressed in stem cells of the central nervous system (CNS)<sup>3</sup> but not in mature CNS cells.<sup>4</sup> Nestin expression is used extensively as a marker for CNS stem cells in the developing nervous system and *in vitro* cultured cells.<sup>5-10</sup> Its transient expression is a critical step in the neural differentiation pathway.<sup>2</sup> Nestin is also expressed in non-neural stem cell populations, such as pancreatic islet progenitors<sup>11-13</sup> and hematopoietic progenitors.<sup>14</sup>

## References

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## Immunocytochemistry Validation

This antibody has been tested for immunocytochemistry using undifferentiated rat cortical stem 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 1X (1:10 dilution) in blocking buffer for 3 hours at room temperature in the dark. Between each step, cells were washed with PBS containing BSA. If a staining volume of 250  $\mu$ L is used, this kit can be used for 20 tests; 100 tests can be done in a staining volume of 50  $\mu$ 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.

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