

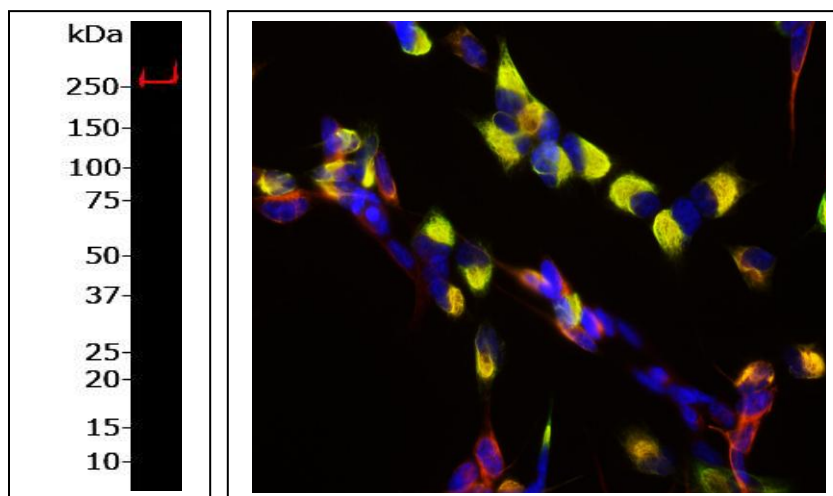
Catalogue# RPCA-Nestin: Rabbit Polyclonal Antibody to the nestin

The Immunogen: Nestin is a member of the class IV intermediate filament protein family which is expressed in neuronal stem cells, which is the origin of the name nestin (1). Nestin was originally identified as a result of the production of a series of monoclonal antibodies directed against epitopes expressed on formalin fixed embryo day 15 rat spinal cord (2). One of these antibodies, called Rat 401, stained fibrous profiles in the developing nervous system, but not in the mature nervous system. By screening bacteriophage expression libraries with Rat 401, Lendahl et al. (1) were able to isolate a cDNA encoding the protein to which Rat 401 antibody bound.

The protein proved to be an unusual member of the intermediate filament family, containing an α -helical region homologous to that found in keratin and neurofilament subunits. The Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC) name for Nestin is NES.

The Nestin protein has a very short N-terminal region in front of the alpha-helical region and a very long and repetitive C-terminal region. The molecular weight of human Nestin as determined by SDS-PAGE mobility is about 240 kDa. However the real molecular weight is considerably less than this, at 177 kDa, the disparity being likely due to the highly charged region of the C-terminal segment. In addition, nestin forms heterodimers with other intermediate filaments, with the class III intermediate filament protein vimentin being its main partner (3).

Nestin is relatively poorly conserved in protein sequence across species boundaries, so that the mouse and human proteins have an overall identity of only 62%. As a result antibodies to the human protein often fail to recognize the rodent homologue and vice versa. Antibodies to nestin are widely used to identify neural stem cells. This antibody was raised in rabbit against recombinant human nestin amino acids 315-630, which has only 55% identity to homologous mouse sequence. RPCA-Nestin therefore recognizes nestin protein in human, but not in rodent.



Left: Western blot of SH-SY5Y homogenate probed with RPCA-Nestin at 1:2,000. A single strong band running at \sim 260 kDa corresponds to full length nestin. **Right:** SH-SY5Y neuroblastoma cells were stained with RPCA-Nestin at 1:5,000 (red) and CPCA-Vim against vimentin (green) at 1: 10,000. Vimentin is main partner of nestin forming heterodimers and further polymerizing to form intermediate filaments. Co-localization of vimentin and nestin in cells was shown in yellow. Blue is DAPI staining of DNA.

Antibody Characteristics: Antibody was raised in rabbit against recombinant human nestin amino acids 315-630 purified from *E. coli*. Antibody is supplied as an aliquot of serum. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: Try at dilutions of 1:1,000-5,000 for immunofluorescence. For western blots try at 1:1,000 -1: 2000. Antibody recognizes a prominent band at ~260 kDa in SH-SY5Y homogenates.

References:

1. Lendahl U, Zimmerman LB and McKay RD. CNS stem cells express a new class of intermediate filament protein. [Cell 60:585-95 \(1990\)](#).

2. Hockfield S and McKay RD. Identification of major cell classes in the developing mammalian nervous system. [J. Neurosci. 5:3310-3328 \(1985\)](#).

3:Chou YH, Khuon S, Herrmann H, Goldman RD. Nestin promotes the phosphorylation-dependent disassembly of vimentin intermediate filaments during mitosis. [Mol Biol Cell 14: 1468-1478 \(2003\)](#).

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

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