



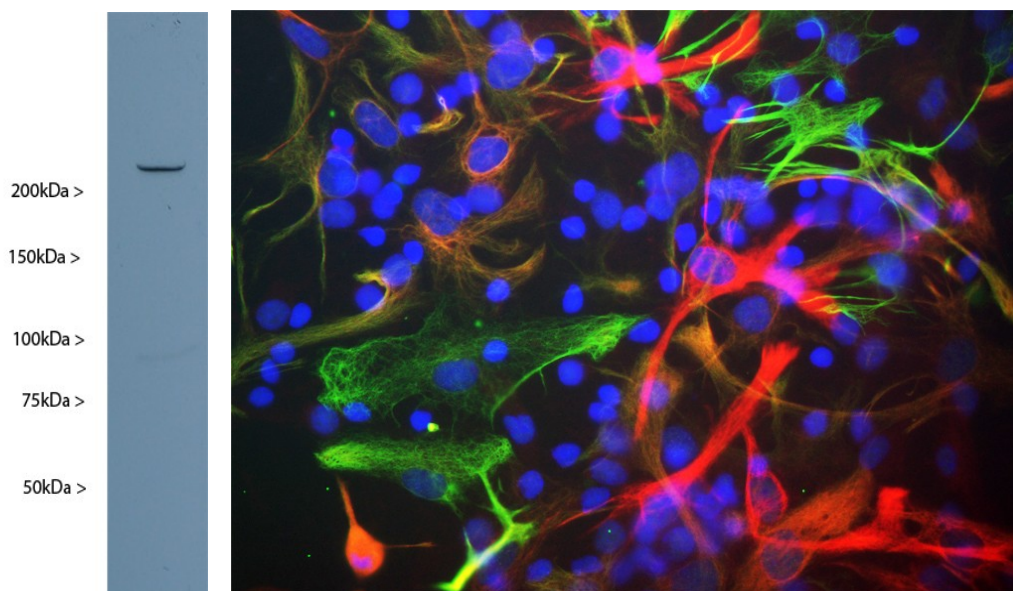
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### **Catalogue MCA-4D11: Mouse Monoclonal Antibody to Nestin (NES)**

**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 alpha-helical region homologous to that found in keratin and neurofilament subunits. The protein was named Nestin as, in the developing nervous system, it is found in neural stem cells. 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.

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. However MCA-4D11 stains both rodent and human Nestin. Antibodies to Nestin are widely used to identify neural stem cells.



**Left:** Western blot in of developing rat brain (P18) homogenate probe with MCA-4D11. A single strong band running at ~240 kDa is seen. **Right:** Mixed cultures of neonatal rat neurons and glia stained with MCA-4D11 (red), chicken antibody to vimentin [CPCA-Vim](#) (green) and DNA (DAPI stain, blue). Astrocytes and neuronal stem cells stain strongly and specifically in a clearly filamentous fashion with the MCA-4D11 antibody. The filamentous staining pattern is as expected as both Nestin and vimentin are components of 10nm filaments. Note that some cells contain Nestin, but do not stain strongly for vimentin and so appear red. Others contain vimentin and not Nestin and so appear green- these are likely to be fibroblastic or endothelial cells. Some cells express both proteins and so appear yellowish. The presence of nestin indicates that the cells are developing astrocytes, neuroblasts or undifferentiated neural stem cells.

**Antibody Characteristics:** The initial challenge was with a preparation of a segment of recombinant human Nestin expressed in bacteria and highly purified. The specific region was amino acids 317 to 630 of the human protein. This is a region which is 55% identical to rat Nestin and 57% identical to mouse Nestin. This antibody is provided as an ascites preparation at more than 1 mg/mL specific antibody content. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

**Suggestions for use:** For immunocytochemistry on cells in tissue culture or in tissue sections, try this antibody at about between about 1:500 using fluorescent secondary antibodies. For immunoblotting 1:5,000 is recommended. Expect to see a band at ~240 kDa and another much weaker at ~90 kDa, apparently a breakdown product of the ~240 kDa band.

**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 \(1985\).](#)

**Antibody Characteristics:** This antibody was raised against recombinant segment of human Nestin which was expressed in *E. coli* with a His-tag and purified by nickel affinity chromatography. The antibody is currently known to stain both human and rodent Nestin.

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

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