

**HA610246**



<b>Product Type:</b>	Recombinant Chimeric Antibody, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	IHC-Fr, IHC-P, WB, IF-Cell
<b>Molecular Wt:</b>	Predicted band size: 207 kDa
<b>Clone number:</b>	PSH07-89

**Description:** Nestin is a major intermediate filament (IF) protein of embryonic central nervous system progenitor cells. It is also a component of the dynamic IF network during muscle development, where it polymerizes with Desmin and Vimentin. Nestin co-assembles with Vimentin or  $\alpha$ -internexin and forms heterodimer coiled-coil molecules which then further assemble into 10 nm IFs. Deletion of the IF consensus rod domain in nestin alters nestin localization in CNS precursor cells and radial glial cells in vivo. Nestin is a marker for neuroepithelial stem cells, glioma cells and tumor endothelial cells during rapid growth. During axon elongation of differentiation neurons, nestin localizes to the growth cones and may play a role in growth cone guidance. In the rat adrenal gland, nestin is expressed by the zona fasciculata and the zona reticularis. Nestin is also expressed by dermatomal cells and by myoblasts during the earliest stages of myogenesis.

**Immunogen:** Recombinant protein within mouse Nestin aa 700-1,000.

**Positive control:** E14.5 mouse embryo tissue, human kidney tissue, mouse kidney tissue, rat kidney tissue, mouse brain (P0) tissue lysates, C2C12, C6.

**Subcellular location:** Cytoplasm, Intermediate filament.

**Database links:** SwissProt: P48681 Human | Q6P5H2 Mouse | P21263 Rat

**Recommended Dilutions:**

IHC-Fr	1:1,000
IHC-P	1:1,000
WB	1:2,000
IF-Cell	1:1,000-1:10,000

**Storage Buffer:** PBS (pH7.4).

**Storage Instruction:** Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

**Hangzhou Huaan Biotechnology Co., Ltd.**

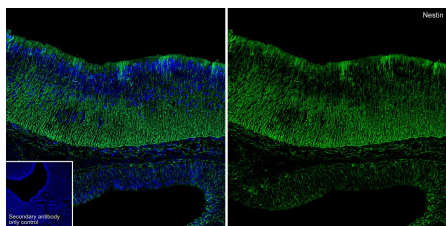
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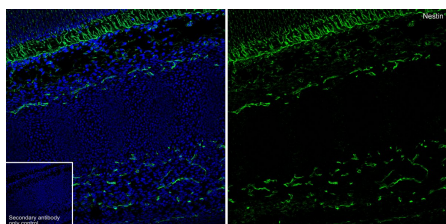
## Images



**Fig1:** Immunofluorescence analysis of frozen E14.5 mouse embryo tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

**The section was not undergone antigen retrieval.**

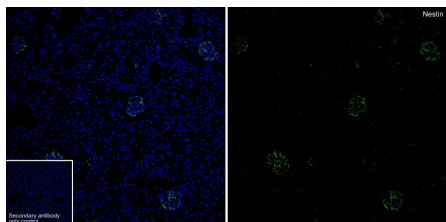
The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA610246, green) at 1/1,000 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rat IgG H&L (iFluor™ 488, HA1133) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).



**Fig2:** Immunofluorescence analysis of frozen E14.5 mouse embryo tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

**The section was not undergone antigen retrieval.**

The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA610246, green) at 1/1,000 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rat IgG H&L (iFluor™ 488, HA1133) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).



**Fig3:** Immunofluorescence analysis of frozen mouse kidney tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

**The section was not undergone antigen retrieval.**

The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA610246, green) at 1/1,000 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rat IgG H&L (iFluor™ 488, HA1133) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).

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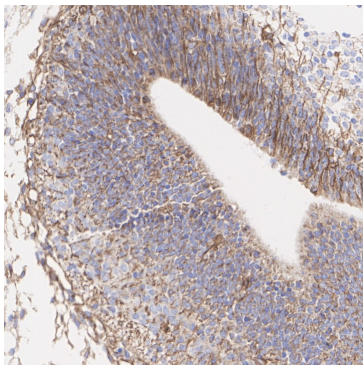
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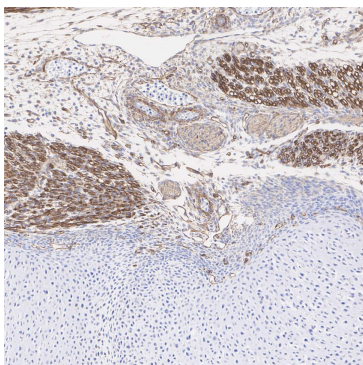
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation



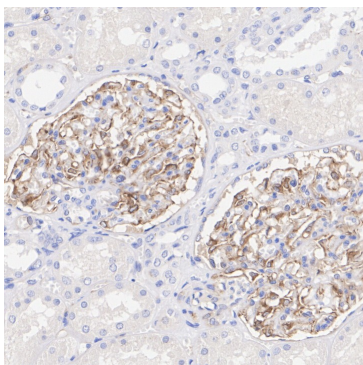
**Fig4:** Immunohistochemical analysis of paraffin-embedded E14.5 mouse embryo tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA610246) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



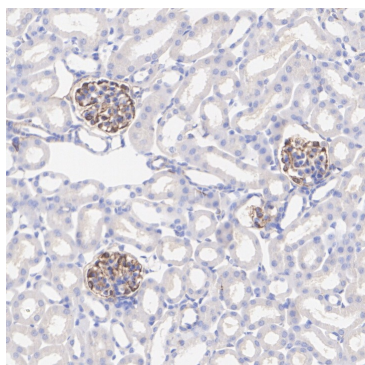
**Fig5:** Immunohistochemical analysis of paraffin-embedded E14.5 mouse embryo tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA610246) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



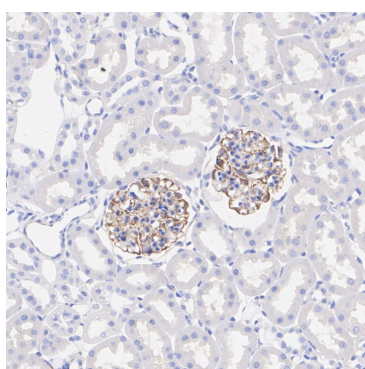
**Fig6:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA610246) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig7:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA610246) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig8:** Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA610246) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

**Fig9:** Western blot analysis of Nestin on mouse brain (P0) tissue lysates with Rat anti-Nestin antibody (HA610246) at 1/2,000 dilution.

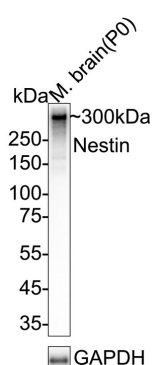
Lysates/proteins at 20 µg/Lane.

Predicted band size: 207 kDa

Observed band size: 300 kDa

Exposure time: 6 seconds; ECL: K1801;

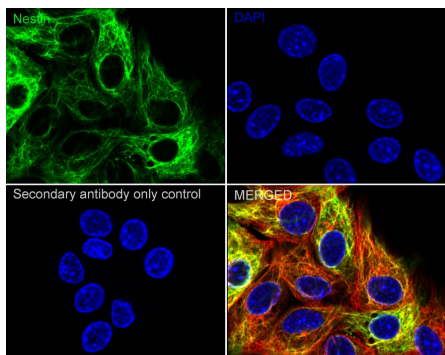
4-20% SDS-PAGE gel.



Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA610246) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rat IgG H&L - HRP Secondary Antibody (HA1023) at 1/5,000 dilution was used for 1 hour at room temperature.



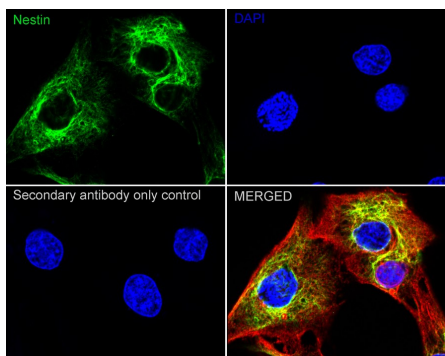
**Fig10:** Immunocytochemistry analysis of C2C12 cells labeling Nestin with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rat anti-Nestin antibody (HA610246) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rat IgG H&L (iFluor™ 488, HA1133) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

**Fig11:** Immunocytochemistry analysis of C6 cells labeling Nestin with Rat anti-Nestin antibody (HA610246) at 1/10,000 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rat anti-Nestin antibody (HA610246) at 1/10,000 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rat IgG H&L (iFluor™ 488, HA1133) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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### Background References

1. Chen H et al. Targeting Nestin(+) hepatic stellate cells ameliorates liver fibrosis by facilitating TbetaRI degradation. J Hepatol. 2021 May
2. Wang J et al. Nestin promotes pulmonary fibrosis via facilitating recycling of TGF-beta receptor I. Eur Respir J. 2022 May

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