Anti-Nestin Antibody [PSH07-89]

HA722919

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IHC-Fr, IF-Tissue, mIHC
Molecular Wt:	Predicted band size: 177 kDa
Clone number:	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 a-internexin and forms heterodimer coiled-coil molecules which then further assemble into 10 nml 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 differen-tiation 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: RD cell lysate, Neuro-2a cell lysate, Mouse brain (P0) tissue lysate, Mouse brain (P7) tissue lysate, Mouse brain tissue lysate, U-87 MG, C2C12, C6, human kidney tissue, mouse kidney tissue, rat kidney tissue, mouse embryonic brain tissue.

Subcellular location: Cytoplasm, Intermediate filament.

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

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:1,000-1:5,000
IHC-P	1:1,000
IHC-Fr	1:500
IF-Tissue	1:500
mIHC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

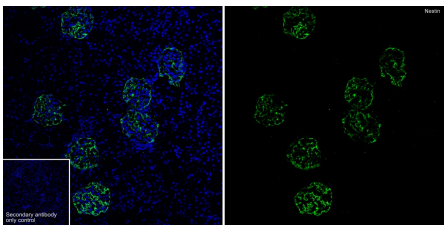

Fig1: Immunofluorescence analysis of frozen rat kidney tissue with Rabbit anti-Nestin antibody (HA722919) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. 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 (HA722919, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

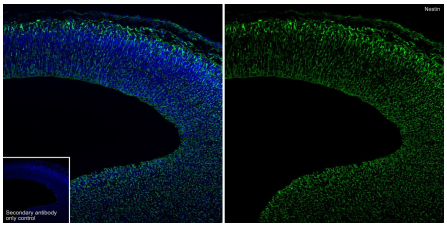

Fig2: Immunofluorescence analysis of frozen mouse embryonic brain tissue with Rabbit anti-Nestin antibody (HA722919) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. 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 (HA722919, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

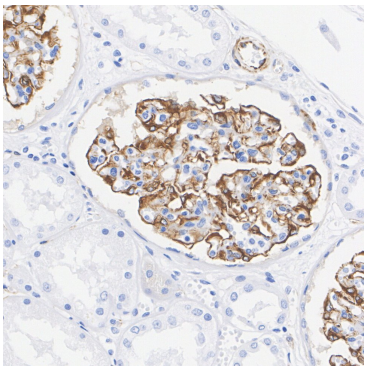

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA722919) 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.

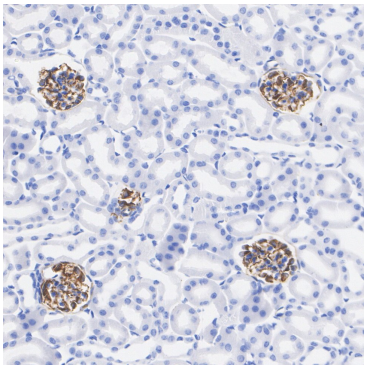

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA722919) 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.

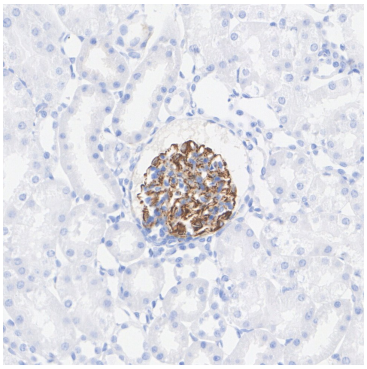

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA722919) 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.

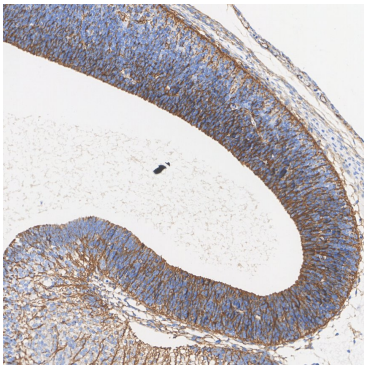

Fig6: Immunohistochemical analysis of paraffin-embedded mouse embryonic brain tissue with Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA722919) 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.

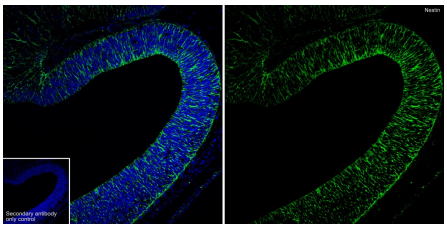

Fig7: Immunofluorescence analysis of paraffin-embedded mouse embryonic brain tissue labeling Nestin with Rabbit anti-Nestin antibody (HA722919) at 1/500 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722919, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

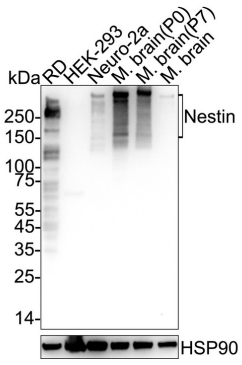

Fig8: Western blot analysis of Nestin on different lysates with Rabbit anti-Nestin antibody (HA722919) at 1/2,000 dilution.

Lane 1: RD cell lysate
Lane 2: HEK-293 cell lysate (negative)
Lane 3: Neuro-2a cell lysate
Lane 4: Mouse brain (P0) tissue lysate
Lane 5: Mouse brain (P7) tissue lysate
Lane 6: Mouse brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 177 kDa
Observed band size: 150-300 kDa

Exposure time: 15 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 (HA722919) at 1/2,000 dilution was used in primary antibody dilution at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

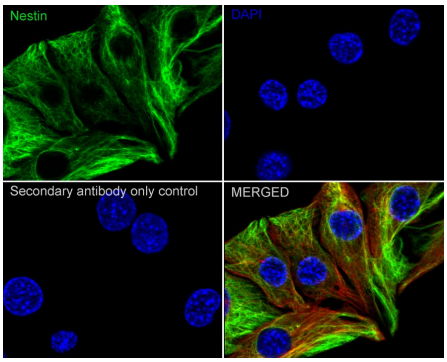

Fig9: Immunocytochemistry analysis of C2C12 cells labeling Nestin with Rabbit anti-Nestin antibody (HA722919) at 1/5,000 dilution.

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 Rabbit anti-Nestin antibody (HA722919) at 1/5,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

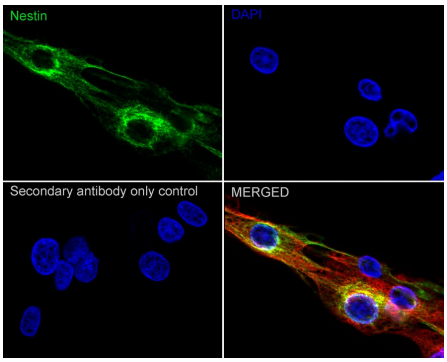

Fig10: Immunocytochemistry analysis of C6 cells labeling Nestin with Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution.

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 Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

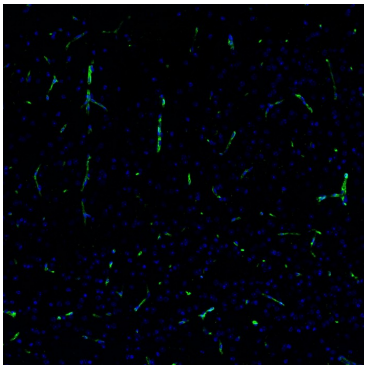

Fig11: mIHC analysis of mouse brain tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-Nestin antibody (HA722919) at 1/1,000 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Background References

1. Yang Y et al. Heightened potency of human pluripotent stem cell lines created by transient BMP4 exposure. Proc Natl Acad Sci U S A 112:E2337-46 (2015).
2. Chen G et al. Human Brat ortholog TRIM3 is a tumor suppressor that regulates asymmetric cell division in glioblastoma. Cancer Res 74:4536-48 (2014).