

Anti-Nestin Antibody [PSH07-89] - BSA and Azide free

HA751196



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IHC-Fr, IF-Tissue, IP
Molecular Wt:	Predicted band size: 207 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 α -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: 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	1:500-1:2,000
IF-Tissue	1:500
IP	Use at an assay dependent concentration.

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.

Orders: 0086-571-88062880

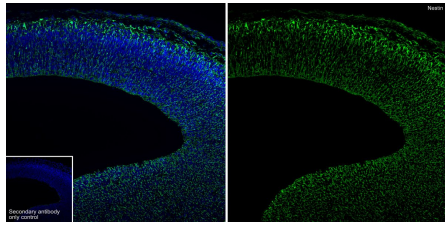
Technical: 0086-571-89986345

Service mail: support@huabio.cn

华安生物
HUABIO
www.huabio.cn

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

Images

**Fig1:** Application: IHC-Fr

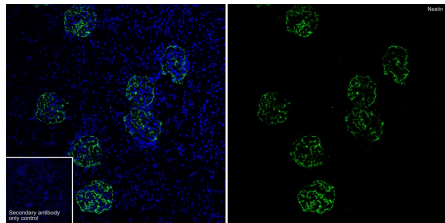
Species: Mouse

Site: E14.5 embryonic brain

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

**Fig2:** Application: IHC-Fr

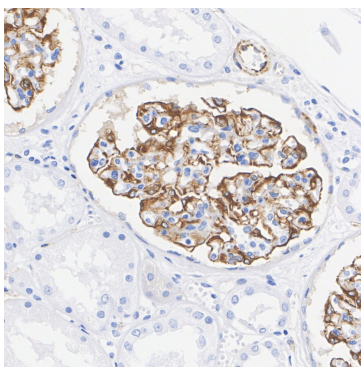
Species: Rat

Site: Kindey

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

**Fig3:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Nestin antibody (HA751196) 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 ddH₂O and PBS, and then probed with the primary antibody (HA751196) 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.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn

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

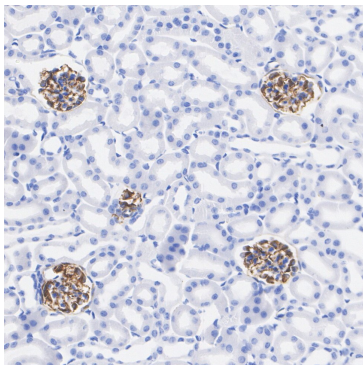


Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Nestin antibody (HA751196) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA751196) 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.

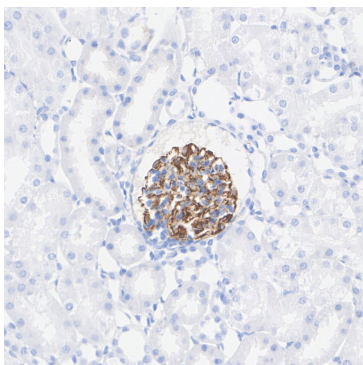


Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Nestin antibody (HA751196) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA751196) 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.

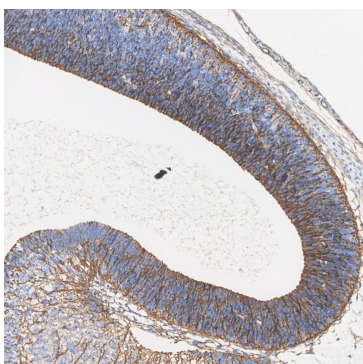
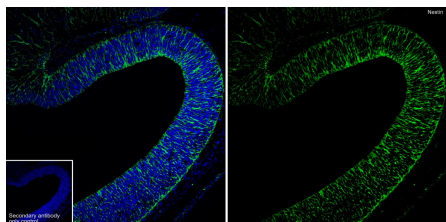


Fig6: Immunohistochemical analysis of paraffin-embedded mouse embryonic brain tissue with Rabbit anti-Nestin antibody (HA751196) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA751196) 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.

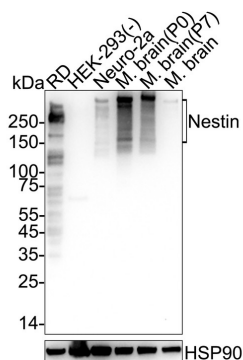
**Fig7:** Application: IF-tissue

Species: Mouse

Site: E14.5 embryonic brain

Sample: Paraffin-embedded section

Antibody concentration: 1/500

Fig8: Western blot analysis of Nestin on different lysates with Rabbit anti-Nestin antibody (HA751196) 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: 177/207 kDa

Observed band size: 150-300 kDa

Exposure time: 15 seconds; ECL: K1801;

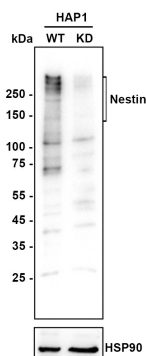
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751196) at 1/2,000 dilution was used in primary antibody dilution (K1803) at 4 °C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig9: Western blot analysis of Nestin on different lysates with Rabbit anti-Nestin antibody (HA751196) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate

Lane 2: HAP1-Nestin KD cell lysate



Lysates/proteins at 10 µg/Lane.

Predicted band size: 177 kDa

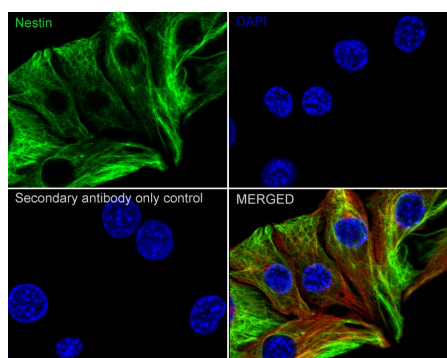
Observed band size: 150-300 kDa

Exposure time: 20 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 (HA751196) at 1/1,000 dilution was used in primary antibody dilution (K1803) at 4 °C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig10: Immunocytochemistry analysis of C2C12 cells labeling Nestin with Rabbit anti-Nestin antibody (HA751196) 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 (HA751196) at 1/5,000 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn

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

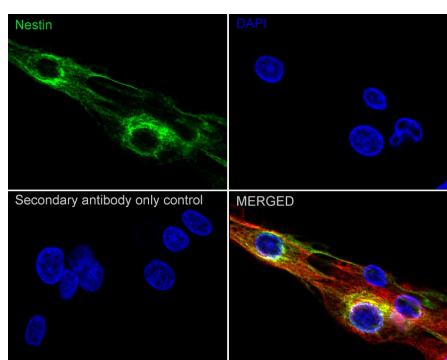


Fig11: Immunocytochemistry analysis of C6 cells labeling Nestin with Rabbit anti-Nestin antibody (HA751196) at 1/1,000 dilution.

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 (HA751196) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

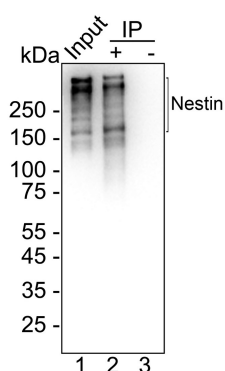


Fig12: Nestin was immunoprecipitated from 0.2 mg C2C12 cell lysate with HA751196 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA751196 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: C2C12 cell lysate (input)

Lane 2: HA751196 IP in C2C12 cell lysate

Lane 3: Rabbit IgG instead of HA751196 in C2C12 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 6 seconds; ECL: K1801

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

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

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUAABIO
www.huabio.cn

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