abcam

Product datasheet

Anti-Nestin antibody [SP103] ab105389

KO VALIDATED Recombinant RabMAb

9 Abreviews 59 References 18 Images

Overview

Product name	Anti-Nestin antibody [SP103]
Description	Rabbit monoclonal [SP103] to Nestin
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-Fr, WB, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human **Predicted to work with:** Dog
Immunogen	Synthetic peptide within Human Nestin aa 1600 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **P48681**
Positive control	IHC-P: Human kidney, ovary, cervix, breast, colon, endometrium, cerebellum, lung, prostate, skin and human melanoma tissue. WB: HeLa cell lysate. Flow Cyt-Intra: SH-SY5Y cells. ICC/IF: SKNSH cells. IHC-Fr: Frozen human kidney tissue sections
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. **This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.60 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein A/G purified

Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP103
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab105389 in the following tested applications.

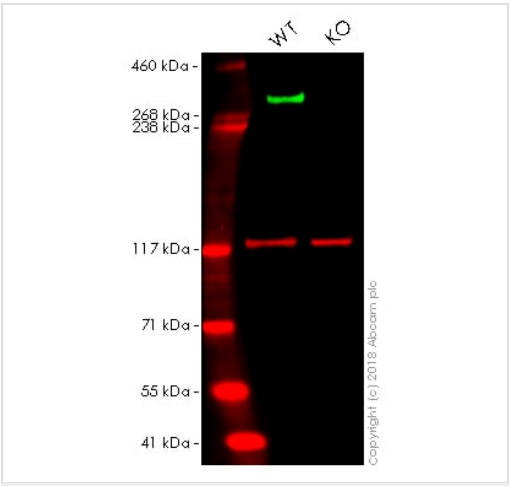
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (2)	1/200.
IHC-Fr		Use a concentration of 5 µg/ml.
WB	★★★★★ (3)	1/100. Predicted molecular weight: 177 kDa.
IHC-P	★★★★★ (4)	1/100. Antigen retrieval: Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min.
Flow Cyt (Intra)		1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function	Required for brain and eye development. Promotes the disassembly of phosphorylated vimentin intermediate filaments (IF) during mitosis and may play a role in the trafficking and distribution of IF proteins and other cellular factors to daughter cells during progenitor cell division. Required for survival, renewal and mitogen-stimulated proliferation of neural progenitor cells.
Tissue specificity	CNS stem cells.
Sequence similarities	Belongs to the intermediate filament family.
Developmental stage	Upon terminal neural differentiation, nestin is down-regulated and replaced by neurofilaments.
Post-translational modifications	Constitutively phosphorylated. This increases during mitosis when the cytoplasmic intermediate filament network is reorganized.

Images



Western blot - Anti-Nestin antibody [SP103] (ab105389)

All lanes : Anti-Nestin antibody [SP103] (ab105389) at 1/100 dilution

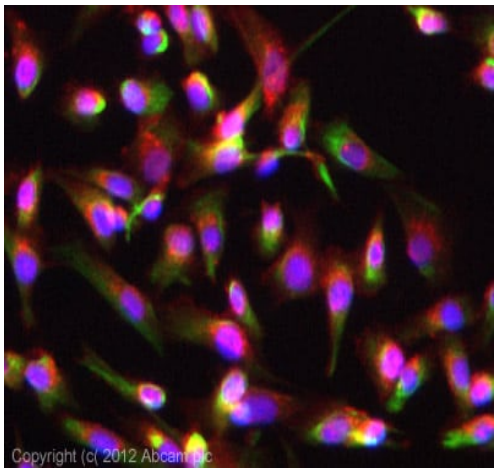
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : NES knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 177 kDa

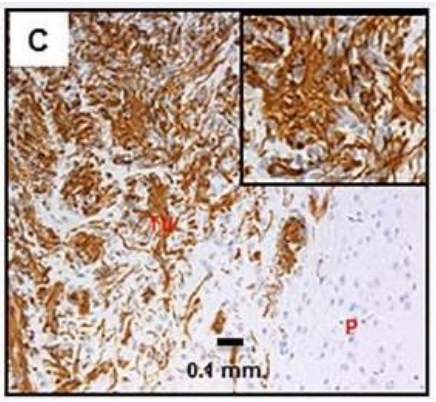
Lanes 1 - 2: Merged signal (red and green). Green - ab105389 observed at 300 kDa. Red - loading control, **ab18058**, observed at 117 kDa.

ab105389 was shown to specifically react with NES in wild-type HAP1 cells as signal was lost in NES knockout cells. Wild-type and NES knockout samples were subjected to SDS-PAGE. Ab105389 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/100 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Nestin antibody [SP103] (ab105389)

ICC/IF image of ab105389 stained SKNSH cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab105389, 1/200 dilution) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

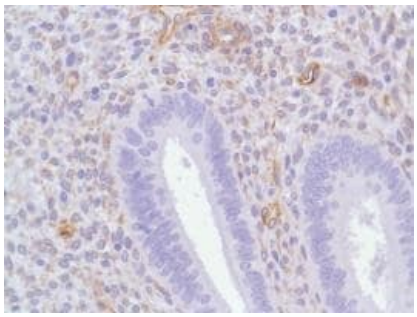
BTIC markers in our CNS-PNET model tumors

Formalin-fixed, paraffin-embedded mouse primitive neural ectodermal tumor tissue was stained for Nestin using ab105389 at 1/30 dilution in immunohistochemical analysis.

Insert x20 magnification.

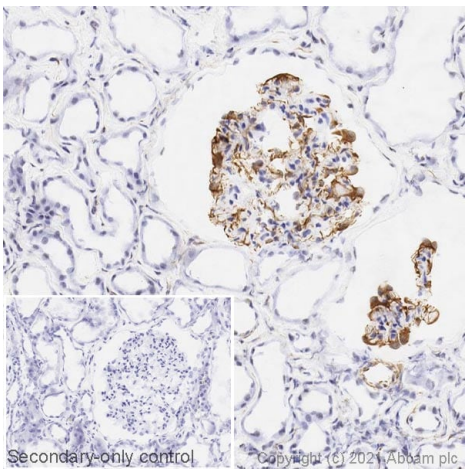
TU-tumor; P-parenchyma; V-ventricle; CP-choroid plexus

(From Figure 3C of Malchenko et al)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human endometrium tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.

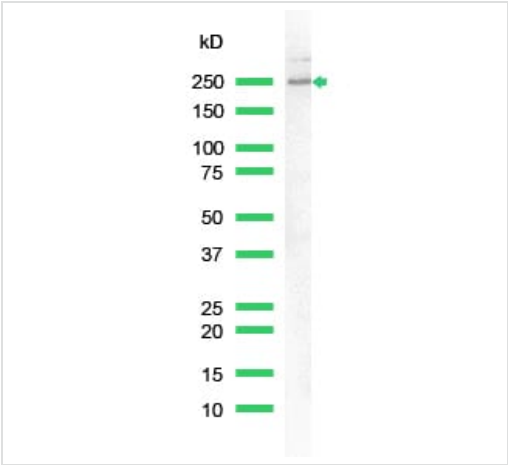


Immunohistochemistry (Frozen sections) - Anti-Nestin antibody [SP103] (ab105389)

IHC image of Nestin staining in a section of frozen normal human kidney* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab105389, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

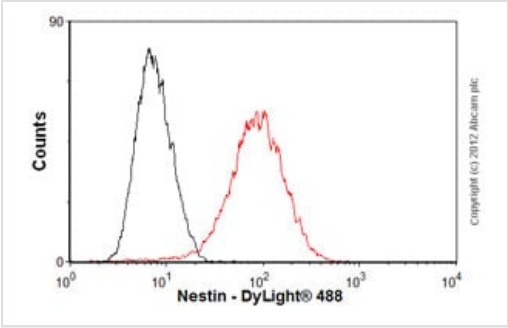
**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Western blot - Anti-Nestin antibody [SP103] (ab105389)

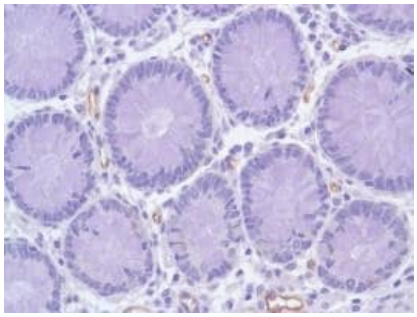
Anti-Nestin antibody [SP103] (ab105389) at 1/100 dilution + HeLa cell lysate

Predicted band size: 177 kDa



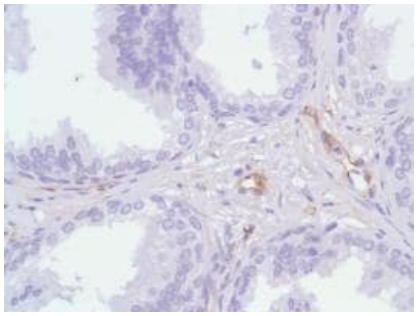
Flow Cytometry (Intracellular) - Anti-Nestin antibody [SP103] (ab105389)

Overlay histogram showing SH-SY5Y cells stained with ab105389 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab105389, 1/50 dilution) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



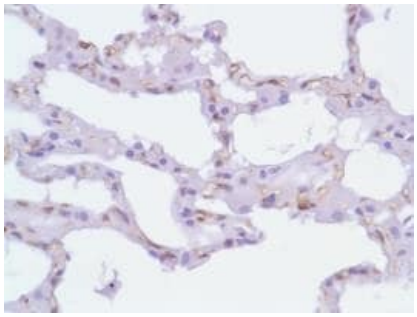
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human colon tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



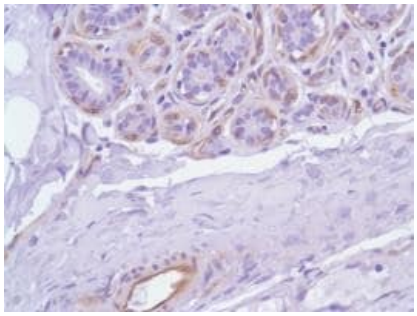
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human prostate tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



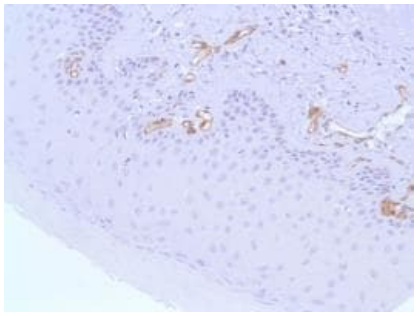
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human lung tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



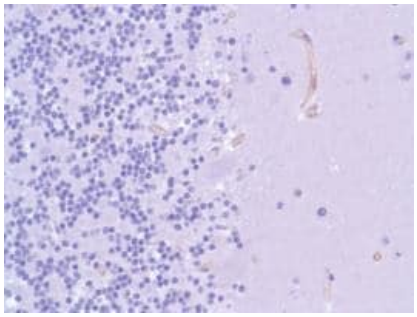
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human breast tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



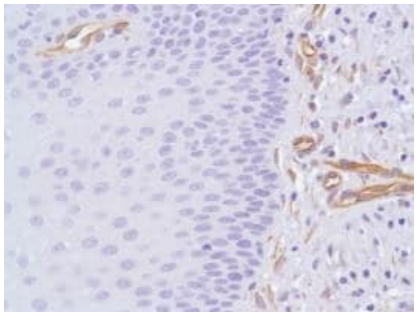
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human skin tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



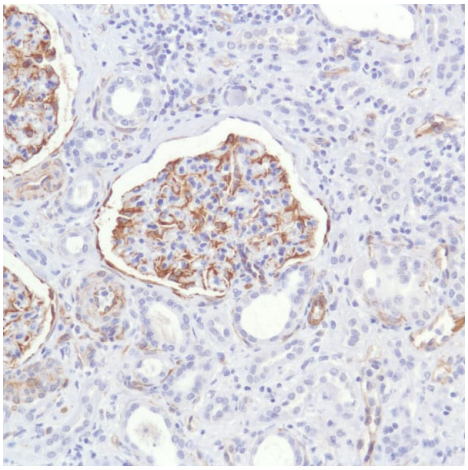
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human cerebellum tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



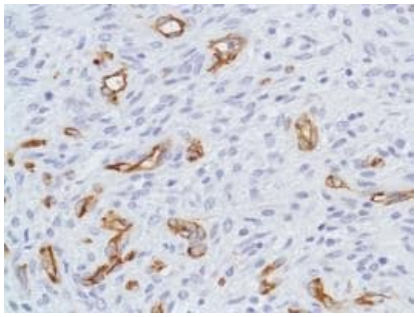
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human cervix tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



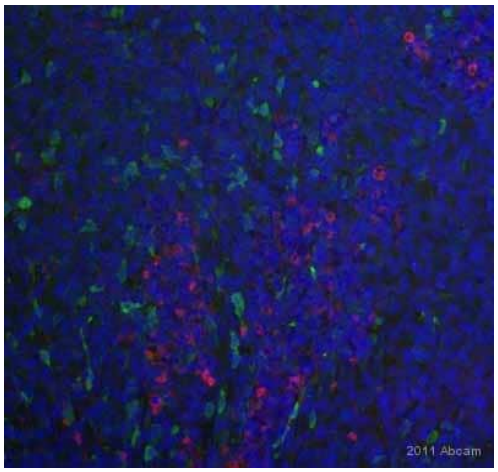
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human kidney tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

Formalin-fixed, paraffin-embedded human ovary tissue stained for Nestin using ab105389 at 1/100 dilution in immunohistochemical analysis.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nestin antibody [SP103] (ab105389)

This image is courtesy of Hongwei Shao by Abreview.

ab105389 staining Nestin in human melanoma tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Tissue was fixed in formaldehyde and a heat mediated antigen retrieval step was performed using Tris EDTA pH 9.0. Samples were then permeabilized using 0.25% Triton X-100, blocked, then incubated with ab105389 at a 1/100 dilution for 18 hours at 4°C. The secondary used was an Alexa-Fluor 594 (red) conjugated donkey anti-rabbit polyclonal used at a 1/100 dilution. (Green) tumor cells, (blue) DAPI.

Anti-Nestin antibody [SP103] (ab105389)

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