

Product Datasheet

Nestin Antibody - BSA Free NB100-1604

Unit Size: 0.25 ml

Store at 4C in the dark.

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NB100-1604

Nestin Antibody - BSA Free

Product Information	
Unit Size	0.25 ml
Concentration	0.3 mg/ml
Storage	Store at 4C in the dark.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgY
Purity	Immunogen affinity purified
Buffer	10mM PBS (0.9% isotonic, w/v, pH 7.2)
Target Molecular Weight	250 kDa

Product Description	
Host	Chicken
Gene ID	10763
Gene Symbol	NES
Species	Human, Mouse, Rat, Baboon, Monkey
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 19481056). Baboon reactivity reported in scientific literature (PMID: 30657788). Monkey reactivity reported in scientific literature (PMID: 30657788). Human reactivity reported in scientific literature (PMID: 33023162).
Marker	Neural Stem Cell Marker
Immunogen	Chickens were immunized with three synthetic peptide/keyhole limpet hemocyanin (KLH) conjugates. These synthetic peptides corresponded to different regions of mouse Nestin (NM_NP_057910), but are shared with the rat (NM_012987, NCBI) protein sequence.
Notes	<p>Chicken products cannot be exported to Canada.</p> <p>Purification Notes After repeated injections, immune eggs were collected, and the IgY fractions were purified from the yolks. These IgY fractions were then affinity-purified using a peptide column, and the concentrations of the eluates adjusted to 300 ug/ml. Finally, equal volumes of each of the three affinity-purified anti-peptide antibodies were mixed, and the preparation was filter sterilized.</p> <p>Storage Notes Store at 4C in the dark. Under these conditions, the antibodies should have a shelf life of at least 12 months (provided they remain sterile). Do not freeze these antibodies unless you want to store them for longer periods of time. Note, however, that each time an antibody preparation is frozen, about half of its binding activity is lost.</p>

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin



Recommended Dilutions

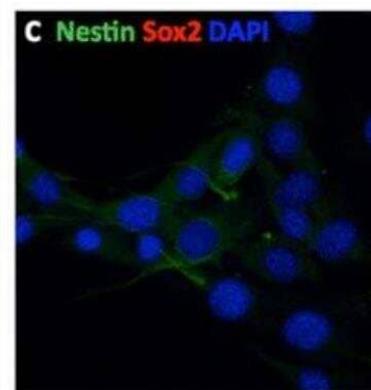
Western Blot 1:10000 - 1:20000, Flow Cytometry, Immunohistochemistry 1:1000 - 1:2000, Immunocytochemistry/ Immunofluorescence 1:1000 - 1:2000, Immunohistochemistry-Paraffin 1:1000 - 1:2000, Immunohistochemistry-Frozen 1:1000 - 1:2000

Images

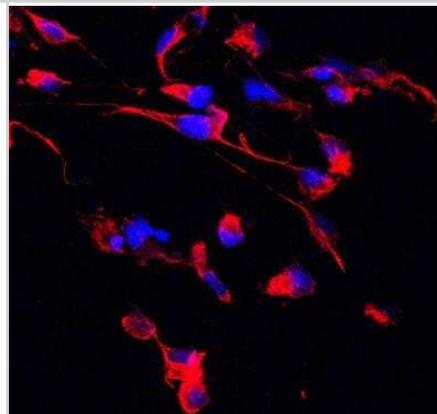
Immunocytochemistry/Immunofluorescence: Nestin Antibody [NB100-1604] - Characterization of MSCmix and NCSCmix isolated from the bone marrow of adult Wnt1-CRE/R26R-LacZ mice. NCSCmix express Nestin (green), Sox2 (red) Scale bar = 20 um. NCSC = neural crest stem cell, MSC = mesenchymal stem cell. Image collected and cropped by CiteAb from the following publication (<https://stemcellres.com/content/6/1/211>) licensed under a CC-BY license.



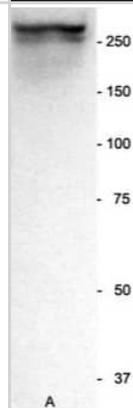
Immunocytochemistry/Immunofluorescence: Nestin Antibody [NB100-1604] - Characterization of MSCmix and NCSCmix isolated from the bone marrow of adult Wnt1-CRE/R26R-LacZ mice. MSCmix are adherent fibroblast-like cells, do not express Sox2 c (red), slightly express Nestin (c) (green). Scale bar = 20 um. MSC mesenchymal stem cell. Image collected and cropped by CiteAb from the following publication (<https://stemcellres.com/content/6/1/211>) licensed under a CC-BY license.



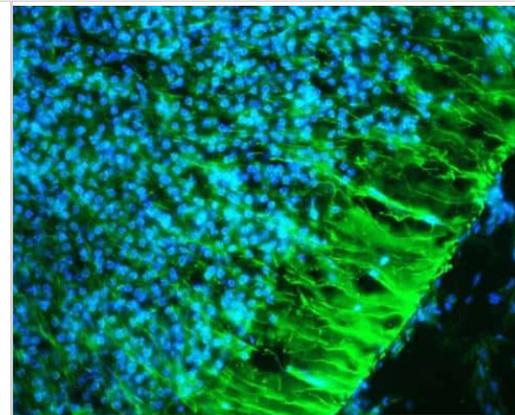
Immunohistochemistry-Frozen: Nestin Antibody [NB100-1604] - Analysis of Nestin in frozen mouse tissue using anti-Nestin antibody. IHC-Fr image submitted by a verified customer review.



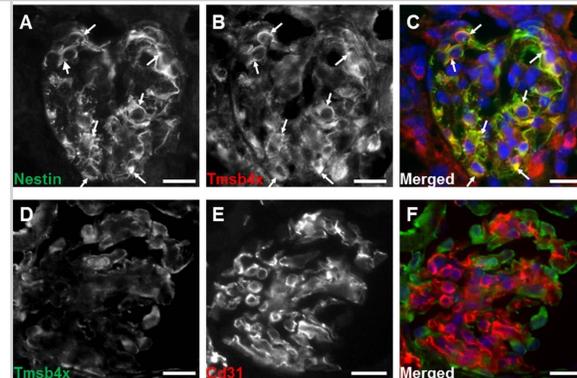
20 ug of total protein from adult mouse brain using NB100-1604 (1:2000)



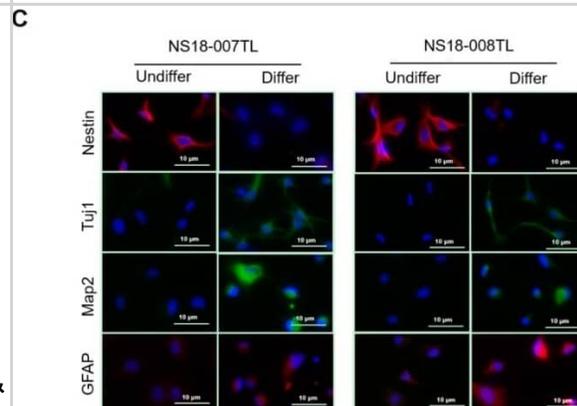
Photomicrograph of a tissue section through the periventricular zone of an e16 mouse brain (4.0% paraformaldehyde-fixed). Green is Nestin immunoreactivity (1:1000 dilution). Blue is DAPI nuclear staining.



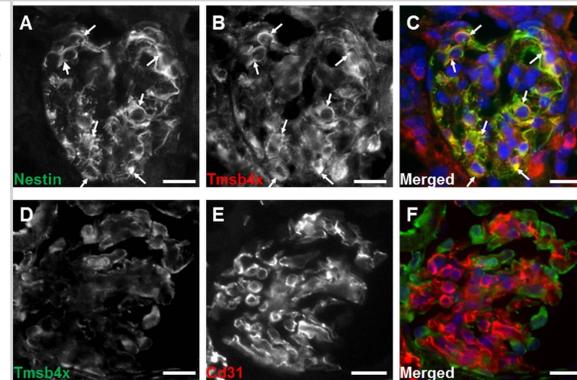
Representative images of nestin (A) and Tmsb4x (B) staining in the mouse adult wild-type glomerulus visualized by fluorescent microscopy. (C) Merged images showing Tmsb4x (red) and nestin (green) staining; areas of colocalization are indicated by arrows. Representative images of Tmsb4x (D) and Cd31 (E) staining in the mouse adult wild-type glomerulus visualized by fluorescent microscopy. (F) Merged images showing Tmsb4x (green) and Cd31 (red) staining. Bar = 20 μ m.



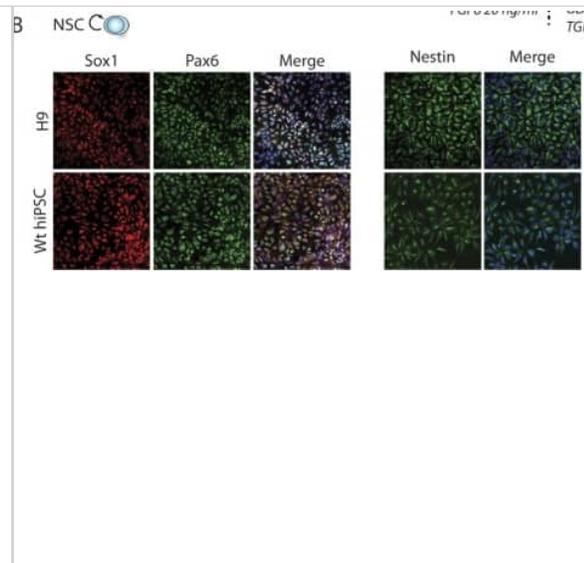
Immunocytochemistry/ Immunofluorescence: Nestin Antibody [NB100-1604] - In vitro proliferation & differentiation of ahMNCs-Clump II. (A) ahMNCs-Clump II were propagated in the culture condition for ahMNCs. The accumulated number of cells of ahMNCs-Clump II (NS18-007TL & 008TL) in comparison with ahMNCs established using previous culture methods (NS14-010TL, NS14-011TL, & NS15-001TL). Days to 1×10^9 cells are indicated. (B) Morphologies of ahMNCs-Clump II under expansion processes are illustrated until third in vitro passage (P3). Scale bar = 50 μ m. (C) After in vitro differentiation, immunofluorescence was applied to ahMNCs-Clump II. Nestin for NSCs, MAP2 & Tuj1 for neurons, & GFAP for astrocytes. Undiffer = before in vitro differentiation; Differ = after in vitro differentiation. Scale bar = 10 μ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30380605>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



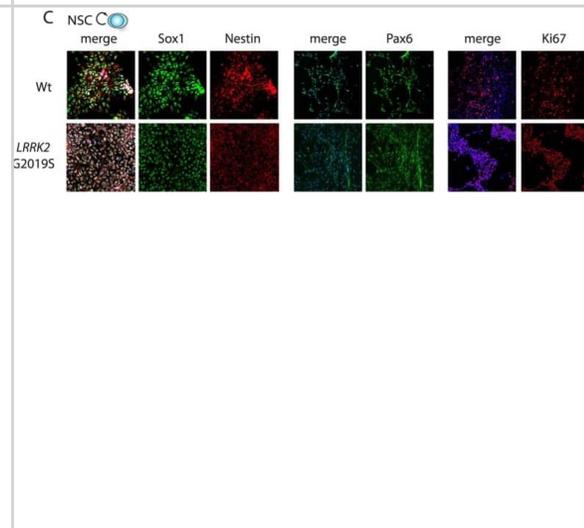
Immunocytochemistry/ Immunofluorescence: Nestin Antibody [NB100-1604] - Representative images of nestin (A) & Tmsb4x (B) staining in the mouse adult wild-type glomerulus visualized by fluorescent microscopy. (C) Merged images showing Tmsb4x (red) & nestin (green) staining; areas of colocalization are indicated by arrows. Representative images of Tmsb4x (D) & Cd31 (E) staining in the mouse adult wild-type glomerulus visualized by fluorescent microscopy. (F) Merged images showing Tmsb4x (green) & Cd31 (red) staining. Bar = 20 μ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/27575556>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



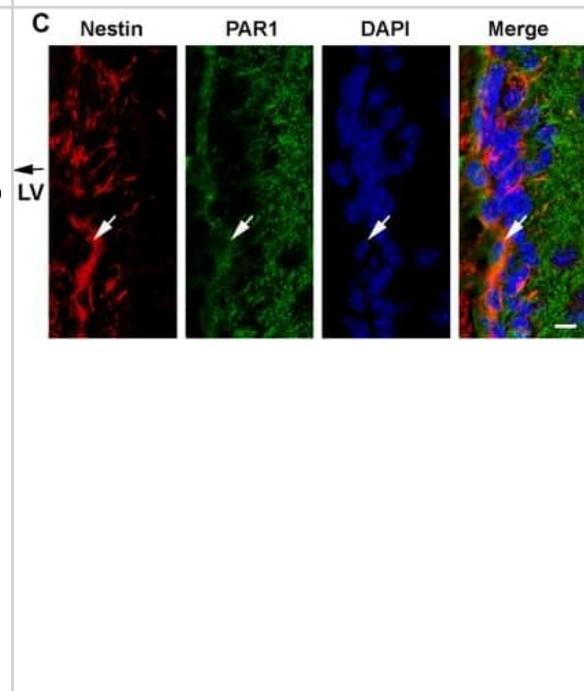
Immunocytochemistry/ Immunofluorescence: Nestin Antibody [NB100-1604] - Dopaminergic neuron derivation from H9 stem cells & hiPSCs. Scheme showing the different steps of the DA neuron derivation protocol (A). Immunolabelings of cultured H9 (top panels) & WT hiPSCs (bottom panels) for different NSCs markers, Sox1, Pax6 & Nestin (B). Immunolabeling of cultured H9 (top panels) & WT hiPSCs (bottom panels) after 3 days of DA progenitor induction for progenitor markers Lmx1A & FoxA2 (C) & after 7 days of induction for immature neurons β III-Tubulin+ & remaining progenitors Sox1+ (D). Immunolabelings of cultured H9 (top panels) & WT hiPSCs (bottom panels) after 21 days of induction for the neuronal marker MAP2 & DA neurons Tyrosine Hydroxylase (TH) (E). Analysis of gene expression levels by qRT-PCR for cultured H9 (top panels) & WT hiPSCs at different time points (NSC, 14 days & 21 days) of the culture (FB = fetal brain) (F,G). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep33377>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Nestin Antibody [NB100-1604] - Neural Stem Cell derivation from WT & LRRK2 G2019S hiPSCs. Haematoxylin & eosin coloration of teratoma-encapsulated tumors generated by flank injection of WT & G2019S hiPSCs cells in Nod/Scid mice. Representative images of the different germ layers: ectoderm (immature squamous epithelium, neural rosettes), mesoderm (primitive cartilage, muscles, fat) & endoderm (primitive gut like epithelium) (A). Scheme showing the different steps of the DA neuron derivation protocol (B). Immunolabelings of cultured WT & LRRK2 G2019S hiPSCs for different NSCs markers after NSCs induction protocol: Sox1, Nestin, Pax6 & Ki67 (C). Analysis of gene expression levels by qRT-PCR for WT & LRRK2 G2019S cultures at the end of the NSCs induction protocol (D). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep33377>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Nestin Antibody [NB100-1604] - PAR1 is expressed by neural stem cells in the sub-ventricular zone (SVZ) of the adult mouse brain. Photomicrographs show immunofluorescence double-labeling for PAR1 with Sox2-positive (A), or PAR1 with Nestin-positive (C) multipotent neural stem cells (NSCs) within the lateral wall of the lateral ventricle (LV). RNAscope was used to identify cells expressing both PAR1 & Sox2 (B), or Nestin (D) RNA in NSCs of the adult SVZ. Arrow indicates an example of a double-labeled cell in each case, with arrowhead indicating a singly labeled cell (Scale bar = 10 μ m). Boxed area in B & D is also shown at higher magnification to visualize double-labeled cells. (E) Histogram shows expression of PAR1 RNA was high in NSCs grown as neurospheres (NS), or when plated on poly-L-lysine coated coverslips as monolayers in stem cell media containing EGF & bFGF. PAR1 RNA expression by NSC monolayers decreased by 87% when EGF & bFGF were removed from the media for 7 DIV promoting stem cell differentiation. (F) Withdrawal of EGF & bFGF to induce NSC monolayer differentiation resulted in a parallel decrease in Nestin RNA expression. (**P < 0.01, ***P < 0.001 Students t-test). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29921916>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

B Di Marco, EE Crouch, B Shah, C Duman, MF Paredes, C Ruiz de Al, EJ Huang, J Alfonso Reciprocal Interaction between Vascular Filopodia and Neural Stem Cells Shapes Neurogenesis in the Ventral Telencephalon Cell Rep, 2020-10-13;33(2):108256. 2020-10-13 [PMID: 33053356]

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Victoria A. Riley, Vijay Shankar, Jennie C. Holmberg, Aidan M. Sokolov, Victoria N. Neckles, Kaitlyn Williams, Rachel Lyman, Trudy F.C. Mackay, David M. Feliciano Tsc2 coordinates neuroprogenitor differentiation iScience 2023-11-14 [PMID: 38107199]

More publications at <http://www.novusbio.com/NB100-1604>



Procedures

Immunohistochemistry Chicken IgY Protocol (NB100-1604)

Citrate Buffer Antigen Retrieval Protocol

Background: Formaldehyde fixation (2% or 4%, or as a component of 10% formalin) produces protein cross-links in tissues that tends to interfere with antibody penetration. This seems to be particularly true of paraffin- embedded formaldehyde-fixed tissue. Since chicken IgY antibodies are larger than rabbit or mouse IgG's, "extra steps" may be necessary to compensate for their larger size.

The citrate-based "antigen retrieval" protocol outlined below has been shown to improve chicken IgY antibody penetration into 4% formalde- hyde-fixed paraffin-embedded sections, and can increase the degree and intensity of immunoreactivity and immunostaining.

Reagents (NOTE: You can use either the Sodium Citrate or Citric Acid Buffers in step #3, below)

"Sodium Citrate Buffer" (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)

Weigh out 2.94 grams of trisodium citrate (dihydrate). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.00 with 1.0 N HCl. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Citric Acid Buffer" (10mM Citric Acid, 0.05% Tween 20, pH 6.0)

Weigh out 1.92 grams of citric acid (anhydrous). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.0 with 1.0 N NaOH. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Phosphate-Buffered Saline" [PBS, 10 mM Sodium phosphate-buffered (pH 7.2) isotonic (0.9%, w/v) saline solution] PBS Tween (0.05% Tween 20 in PBS)
Ethanol (80%, 90%, 95%, 100%) diluted with water

Xylene

Procedure (for use with paraffin-embedded sections):

- 1 Deparaffinize tissue sections in 2 changes of xylene (5 minutes each).
2. Hydrate in 2 changes of 100% ethanol (3 minutes each), 95% ethanol (1 minute), 90% ethanol (1 minute), 80% ethanol (1 minute). Rinse in distilled water.
3. Pre-heat steamer or water bath with staining dish containing either Sodium Citrate Buffer or Citrate Buffer. Wait until temperature reaches 95-100 degrees C.

NOTE: Microwave or pressure cooker can be used as an alternative as a heating source.

4. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes (optimal incubation times will vary).
5. Remove the staining dish, and allow it to cool to room temperature (for 20 minutes or so).

6. Rinse sections in PBS Tween twice for 2 minutes each time.

NOTE: The remainder of this protocol is meant to be a suggestion, and can be substituted with your regular immunostaining protocol.

7. Block sections for 30 minutes with Blocking buffer diluted 1:10 with water.

8. Incubate sections with primary antibody at appropriate dilution in antibody dilution buffer overnight at 4 degrees C. Since chicken IgY antibodies are larger than mammalian IgG's, this overnight incubation allows more time for antibody penetration into tissue sections.

9. Rinse sections with PBS Tween 20 twice for 5 minutes each time.

10. Incubate sections with labeled secondary antibody (see NOTE, below) at appropriate dilution (for one hour at room temperature) in a 1:100 dilution of blocking buffer (diluted in PBS).

11. Rinse with PBS Tween 20 for three times for 5 minutes each time.

NOTE: This protocol may use HRP- or fluorescently-labeled secondary antibodies produced in goats or rabbits.

References:

1. Shi SR, Chaiwun B, Young L, Cote RJ, Taylor CR. (1993). Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical demonstration of androgen receptor in formalin-fixed paraffin sections. *J Histochem Cytochem* 41 (11): 1599-1604.
2. Kanai K, Nunoya T, Shibuya K, Nakamura T, Tajima M (1998). Variations in effectiveness of antigen retrieval pretreatments for diagnostic immunohistochemistry. *Res Vet Sci* 64 (1): 57-61.
3. Brown RW, Chirala R. (1995). Utility of microwave-citrate antigen retrieval in diagnostic immunohistochemistry. *Mod Pathol* 8 (5): 515-20.
4. Morgan JM, Navabi H, Schmid KW, Jasani B (1994). Possible role of tissue-bound calcium ions in citrate-mediated high-temperature antigen retrieval. *J Pathol* 174 (4): 301-7.
5. Pellicer EM, Sundblad A (1994). Antigen retrieval by microwave oven with buffer of citric acid. *Medicina (B Aires)*. 54 (2): 129-32.
6. Shi SR, Chaiwun B, Young L, Cote RJ, Taylor CR (1993). Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical demonstration of androgen receptor in formalin-fixed paraffin sections. *J Histochem Cytochem* 41 (11): 1599-604.



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BAF010	Goat anti-Chicken IgY Secondary Antibody [Biotin]
NB7276	Goat anti-Chicken IgM Heavy Chain Secondary Antibody
NBP2-48575PEP	Nestin Recombinant Protein Antigen
236-EG-200	EGF [Unconjugated]

Limitations

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