**Product Datasheet**

**Nestin Antibody**  
**NB100-1604**

Unit Size: 0.25 ml  
Store at 4°C in the dark.

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# NB100-1604
Nestin Antibody

## Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.3 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at 4°C in the dark.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgY</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>10mM Sodium PBS (0.9% isotonic, w/v, pH 7.2)</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>250 kDa</td>
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## 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).

### 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
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 μg/ml. Finally, equal volumes of each of the three affinity-purified anti-peptide antibodies were mixed, and the preparation was filter sterilized.

Storage Notes
Store at 4°C 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.

## Product Application Details

### Applications
Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin

### Recommended Dilutions
Application Notes

Each of the three antibodies were analyzed by immunohistochemistry (1:20000) using fluorescein-labeled goat anti-chicken IgY (1:500) as the secondary reagent. Use in Immunohistochemistry on frozen sections was reported in a scientific publication (PMID: 23741377). See publications for use in ICC/IF. For WB, use only nitrocellulose membranes, and not PVDF membranes.

Images

Immunocytochemistry/Immunofluorescence: Nestin Antibody [NB100-1604] - Immunocytochemistry showing nestin immunoreactivity (GREEN) in A7r5 neuroblastoma cells in culture. BLUE is DAPI nuclear staining.

Immunohistochemistry-Paraffin: Nestin Antibody [NB100-1604] - Photomicrograph of a paraffin-embedded tissue section through an adult dentate gyrus of the hippocampal formation from a paraformaldehyde-fixed (4%) mouse brain. RED shows nestin immunoreactivity as visualized with a Texas Red goat anti-chicken IgY antibody. Green is staining of the granule cells; BLUE is DAPI nuclear staining.

Immunocytochemistry/Immunofluorescence: Nestin Antibody [NB100-1604] - Photomicrograph of 3T3 cells in culture. Nestin immunoreactivity (RED staining); GREEN is beta-Tubulin 3 staining using a rabbit antibody; BLUE is DAPI nuclear staining.

<table>
<thead>
<tr>
<th>Publications</th>
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<tbody>
<tr>
<td>Zhu, S; Liu, W; Ding, H; Cui, H; Yang, L; BMP4 and Neuregulin regulate the direction of mouse neural crest cell differentiation. Exp Ther Med. May 1 2019 12:00AM [PMID: 31007733] (ICC/IF, Mouse).</td>
</tr>
</tbody>
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More publications at [http://www.novusbio.com/NB100-1604](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% formaldehyde-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:


Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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