

# Product Datasheet

## Netrin-1 Antibody - BSA Free NB100-1605

Unit Size: 0.5 ml

Store at 4C in the dark.

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**NB100-1605**

Netrin-1 Antibody - BSA Free

Product Information	
Unit Size	0.5 ml
Concentration	0.1 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	67 kDa

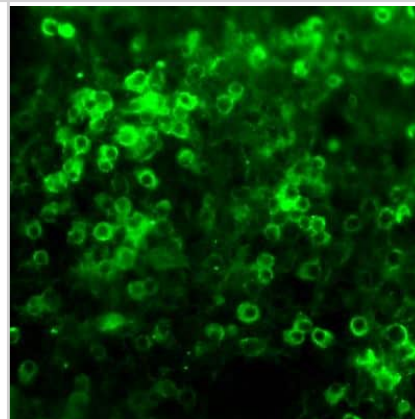
Product Description	
Host	Chicken
Gene ID	9423
Gene Symbol	NTN1
Species	Human, Mouse, Rat
Marker	Neuron Cell Marker
Immunogen	Chickens were immunized with a synthetic peptide-keyhole limpet hemocyanin (KLH) conjugate. This synthetic peptide corresponded to Netrin-1, but was shared between the human (O_095631, NCBI) and mouse (O_09118, NCBI) sequences.
Notes	<p>Chicken products cannot be exported to Canada.</p> <p><b>Purification Notes</b> After repeated injections into the hens, immune eggs were collected, and the IgY fractions were purified from the yolks. These IgY fractions were then affinity-purified using a peptide column, the concentrations of the eluate adjusted to 100 ug/ml, and the preparation filter-sterilized.</p> <p><b>Storage Notes</b> 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, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot, Immunohistochemistry 1:1000-1:2000, Immunocytochemistry/ Immunofluorescence 1:1000-1:2000, Immunohistochemistry-Paraffin 1:1000-1:2000, Immunohistochemistry-Frozen
Application Notes	Use in Immunohistochemistry-Frozen reported in scientific literature (PMID 26482371). Use in Western Blot reported in scientific literature (PMID 20628609).



## Images

Immunocytochemistry/Immunofluorescence: Netrin-1 Antibody [NB100-1605] - Neurosphere (organoid) cultures of e13 mouse brain were cultured for 2 weeks, and then paraformaldehyde (2%) fixed. After extensive washing, the cultures were incubated with Netrin-1 antibody (1:500 dilution), washed, and then treated with fluorescein-labeled goat anti-chicken IgY (1:500 dilution).



## Publications

Zhang H, Wang L, Cui J et al. Maintaining hypoxia environment of subchondral bone alleviates osteoarthritis progression *Science advances* 2023-04-05 [PMID: 37018403]

B Tavora, T Mederer, KJ Wessel, S Ruffing, M Sadjadi, M Missmahl, BN Ostendorf, X Liu, JY Kim, O Olsen, AL Welm, H Goodarzi, SF Tavazoie Tumoural activation of TLR3-SLIT2 axis in endothelium drives metastasis *Nature*, 2020-09-30;586(7828):299-304. 2020-09-30 [PMID: 32999457]

Cuesta, S, Restrepo-Lozano, J M Et al. DCC-related developmental effects of abused- versus therapeutic-like amphetamine doses in adolescence. *Addict Biol* 2020-07-01 [PMID: 31192517] (ICC/IF, Sheep, Bovine, Porcine, Mouse)

Reynolds LM, Pokinko M, Torres-Berrio A et al. DCC Receptors Drive Prefrontal Cortex Maturation by Determining Dopamine Axon Targeting in Adolescence. *Biol. Psychiatry* 2018-01-15 [PMID: 28720317] (WB, Mouse)

Salameh S, Nouel D, Flores C, Hoops D. An optimized immunohistochemistry protocol for detecting the guidance cue Netrin-1 in neural tissue. *MethodsX* 2018-12-13 [PMID: 29276694] (IHC-P, Mouse)

Wu CH, Yuan XC, Gao F et al. Netrin-1 Contributes to Myelinated Afferent Fiber Sprouting and Neuropathic Pain. *Mol. Neurobiol.* 2015-10-19 [PMID: 26482371] (IHC-Fr, Rat)

Tallman MS, Kim HT, Montesinos P et al. Does microgranular variant morphology of acute promyelocytic leukemia independently predict a less favorable outcome compared with classical M3 APL? A joint study of the North American Intergroup and the PETHEMA Group. *Blood* 2010-12-16 [PMID: 20858857] (Human)

Manitt C, Labelle-Dumais C, Eng C et al. Peri-pubertal emergence of UNC-5 homologue expression by dopamine neurons in rodents. *PLoS One* 2010-07-08 [PMID: 20628609] (ICC/IF, IF/IHC, WB, Rat)

Manitt C, Mimee A, Eng C et al. The Netrin Receptor DCC Is Required in the Pubertal Organization of Mesocortical Dopamine Circuitry. *J Neurosci*;31(23):8381-94. 2011-06-08 [PMID: 21653843]

## Procedures

### Immunohistochemistry Chicken IgY Protocol (NB100-1605)

Immunohistochemistry Chicken IgY Protocol (NB100-1605):

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:

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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### **Products Related to NB100-1605**

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BAF010	Goat anti-Chicken IgY Secondary Antibody [Biotin]
NB7276	Goat anti-Chicken IgM Heavy Chain Secondary Antibody
NBP2-31640PEP	Netrin-1 Recombinant Protein Antigen
DVE00	VEGF [HRP]

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### **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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