

# Product Datasheet

## RBFOX3/NeuN Antibody - BSA Free NBP2-10491-0.5ml

Unit Size: 0.5 ml

Store at 4C in the dark.

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[technical@novusbio.com](mailto:technical@novusbio.com)

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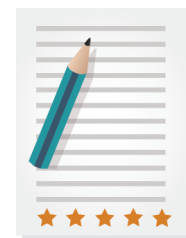
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Updated 3/25/2025 v.20.1

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**NBP2-10491-0.5ml**

RBFOX3/NeuN 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	10 mM PBS (0.9% isotonic, w/v, pH 7.2).
Target Molecular Weight	33.8 kDa

Product Description	
Host	Chicken
Gene ID	146713
Gene Symbol	RBFOX3
Species	Human, Mouse, Rat
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:33754020). Predicted cross-reactivity based on sequence identity: Gorilla (100%), Gibbon (100%), Marmoset (100%).
Marker	Neuron Cell Marker
Immunogen	Hens were immunized with a synthetic peptide-keyhole limpet hemocyanin (KLH) conjugate. This synthetic peptide corresponded to RBFOX3/NeuN, but was shared between the human NP_001076044, NCBI) and mouse (NP_001020102, 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	Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunohistochemistry-Frozen (Negative)
Recommended Dilutions	Flow Cytometry, Immunohistochemistry 1:1000 - 1:2000, Immunocytochemistry/ Immunofluorescence 1:1000-1:2000, Immunohistochemistry-Paraffin 1:1000 - 1:2000, Immunohistochemistry-Frozen (Negative)



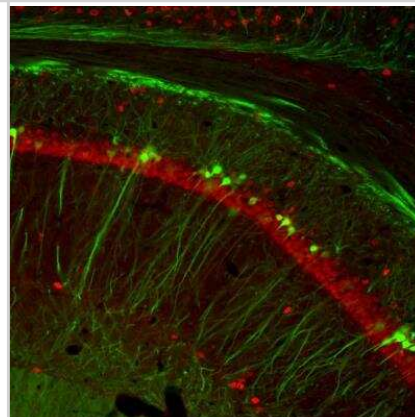
**Application Notes**

Use in Flow reported in scientific literature (PMID:33754020).

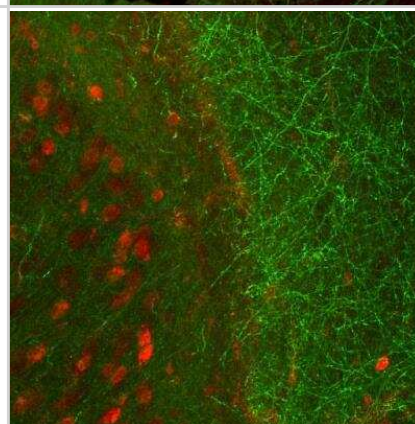
IHC-Fr is not recommended for use as multiple customers have reported lack of signal using this antibody in frozen sections

**Images**

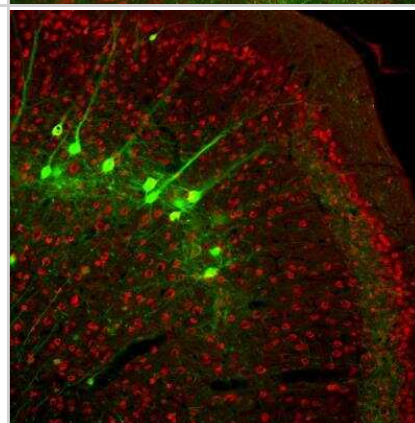
Immunocytochemistry/Immunofluorescence: RBFOX3/NeuN Antibody [NBP2-10491] - Immunohistochemical photomicrograph of adult mouse hippocampal formation stained for beta-III Tubulin rabbit antibody (GREEN) and RBFOX3/NeuN chicken antibody (RED).



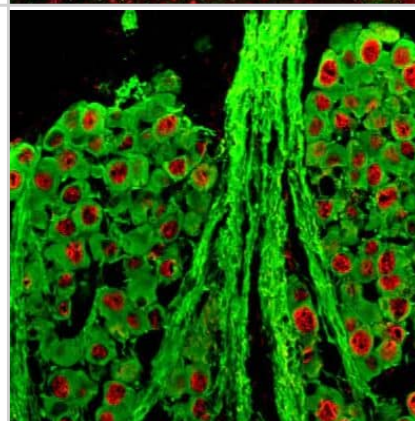
Immunocytochemistry/Immunofluorescence: RBFOX3/NeuN Antibody [NBP2-10491] - Immunohistochemical photomicrograph of adult mouse cerebral cortex stained for beta-III Tubulin rabbit antibody (GREEN) and RBFOX3/NeuN chicken antibody (RED).



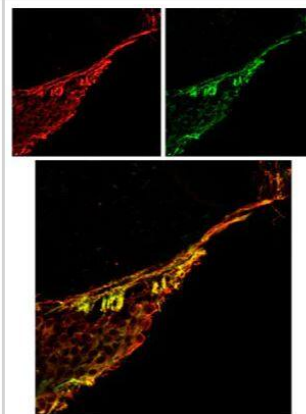
Immunocytochemistry/Immunofluorescence: RBFOX3/NeuN Antibody [NBP2-10491] - Immunohistochemical photomicrograph of adult mouse cerebral cortex stained for beta-III Tubulin rabbit antibody (GREEN) and RBFOX3/NeuN chicken antibody (RED).



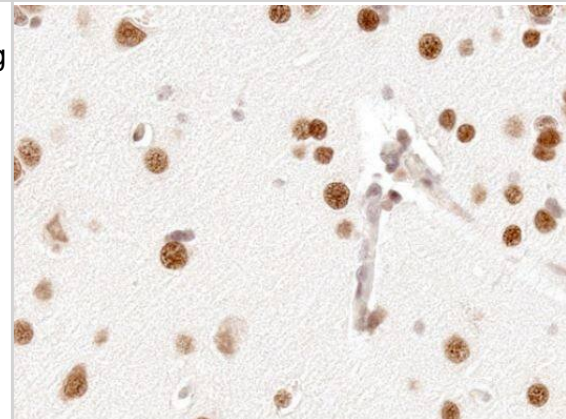
Immunohistochemical photomicrograph of adult auditory ganglion stained for beta-III Tubulin (Rabbit antibody, green; 1:500 dilution) and RBFOX3/NeuN (NBP2-10491, red, 1:1000 dilution).



Immunohistochemical photomicrograph of adult Organ of Corti stained for beta-III Tubulin (green; 1:500 dilution) and RBFOX3/NeuN (NBP2-10491, red, 1:1000 dilution).



Immunohistochemical photomicrograph of neurons in cerebral cortex from a patient with Alzheimer's Disease, paraffin-embedded section using NBP2-10491 (1:1000 dilution). HRP-labeled goat anti-chicken IgY used as secondary antibody (1:1000 dilution).



## Publications

Alexander Popov, Nadezda Brazhe, Kseniia Morozova, Konstantin Yashin, Maxim Bychkov, Olga Nosova, Oksana Sutyagina, Alexey Brazhe, Evgenia Parshina, Li Li, Igor Medyanik, Dmitry E Korzhevskii, Zakhar Shenkarev, Ekaterina Lyukmanova, Alexei Verkhatsky, Alexey Semyanov Mitochondrial malfunction and atrophy of astrocytes in the aged human cerebral cortex. *Nature communications* 2023-12-19 [PMID: 38104196]

Qin L, Vetreno RP, Crews FT NADPH oxidase and endoplasmic reticulum stress is associated with neuronal degeneration in orbitofrontal cortex of individuals with alcohol use disorder *Addiction biology* 2023-01-01 [PMID: 36577732] (IHC-P, Human)

Qiu AW, Huang DR, Li B Et al. IL-17A injury to retinal ganglion cells is mediated by retinal MULLer cells in diabetic retinopathy *Cell death & disease* 2021-11-08 [PMID: 34750361] (ICC/IF, Mouse)

Vetreno RP, Qin L, Coleman LG, Crews FT Increased Toll-like Receptor-MyD88-NF kappa B-Proinflammatory neuroimmune signaling in the orbitofrontal cortex of human alcohol use disorder *Alcoholism, clinical and experimental research* 2021-08-20 [PMID: 34415075] (ICC/IF, Human)

Chen SJ, Chen LH, Yeh YM et al. Targeting lysosomal cysteine protease cathepsin S reveals immunomodulatory therapeutic strategy for oxaliplatin-induced peripheral neuropathy *Theranostics* 2021-03-04 [PMID: 33754020] (FLOW, Mouse)

## Procedures

### Immunohistochemistry Chicken IgY Protocol (NBP2-10491)

Immunohistochemistry Chicken IgY Protocol (NBP2-10491):

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.





### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **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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