Product Datasheet

RBFOX3/NeuN Antibody - BSA Free
NBP1-77686

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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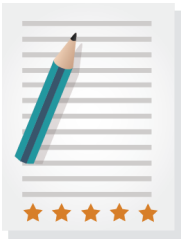
Updated 10/23/2024 v.20.1



NBP1-77686

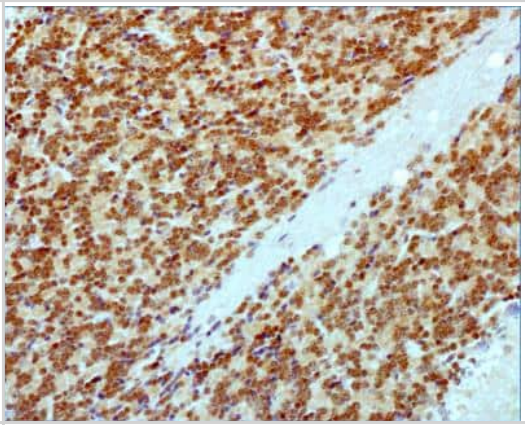
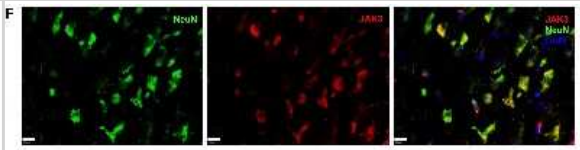
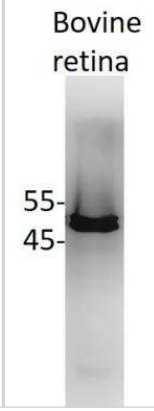
RBFOX3/NeuN Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	33.8 kDa

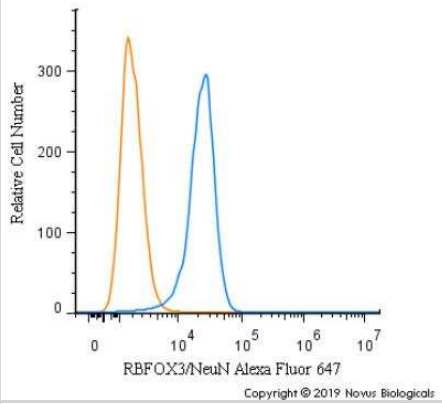
Product Description	
Host	Rabbit
Gene ID	146713
Gene Symbol	RBFOX3
Species	Human, Mouse, Rat, Porcine, Bovine
Reactivity Notes	Bovine and porcine reactivity reported from a verified customer review.
Marker	Neuronal Marker
Immunogen	A synthetic peptide made to an internal region of the human RBFOX3/NeuN protein (within residues 20-100). [Swiss-Prot A6NFN3]

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunohistochemistry Free-Floating
Recommended Dilutions	Western Blot reported in scientific literature (PMID 26581336), Flow Cytometry, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50 - 1:100, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature (detailed protocol in PMID 22771622), Immunohistochemistry Free-Floating reported in scientific literature (detailed protocol in PMID 22771622)
Application Notes	In ICC, nuclear staining was observed in U2OS cells. In IHC-P, staining was observed in the nuclei of mouse brain cells. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. IHC-Fr / IHC-FrFl application reported at 1:100 dilution with detailed protocol in PMID 22771622.

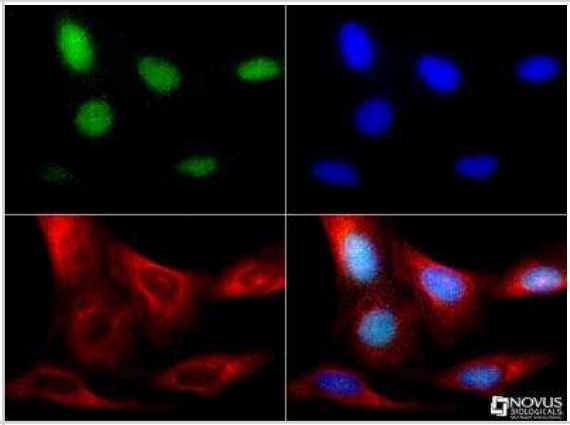
Images

Immunohistochemistry-Paraffin: RBFOX3/NeuN Antibody [NBP1-77686] - IHC analysis of formalin fixed paraffin embedded tissue section from hippocampus of mouse brain with NeuN antibody at 1:200 dilution. The antibody generated a strong nuclear with weak cytoplasmic staining in NeuN reactive cells.	
Immunohistochemistry-Paraffin: RBFOX3/NeuN Antibody [NBP1-77686] - JAK3 colocalized with neurons labeled with NeuN. Image collected and cropped by CiteAb from the following publication (https://journal.frontiersin.org/article/10.3389/fneur.2017.00363/full), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: RBFOX3/NeuN Antibody [NBP1-77686] - Neuro2a cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-RBFOX3/NeuN Antibody NBP1-77686 at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Western Blot: RBFOX3/NeuN Antibody [NBP1-77686] - Western blot image submitted by a verified customer review.	

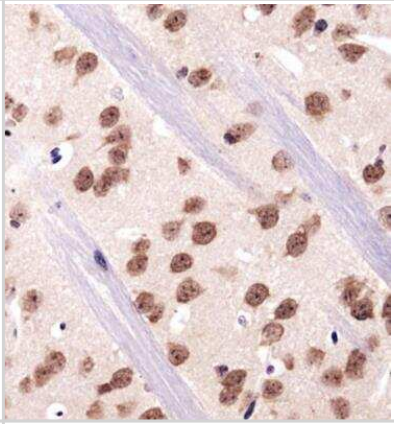
Flow Cytometry: RBFOX3/NeuN Antibody [NBP1-77686] - An intracellular stain was performed on Hek293 cells with NBP1-77686AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



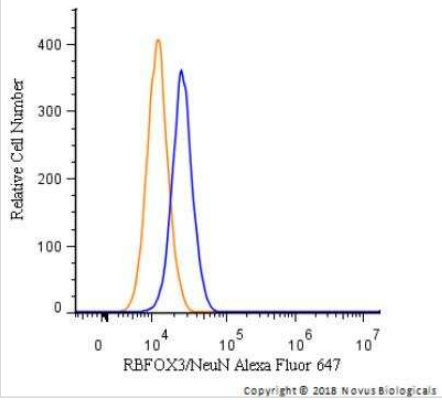
Immunocytochemistry/Immunofluorescence: RBFOX3/NeuN Antibody [NBP1-77686] - NeuN antibody was tested in U2OS cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



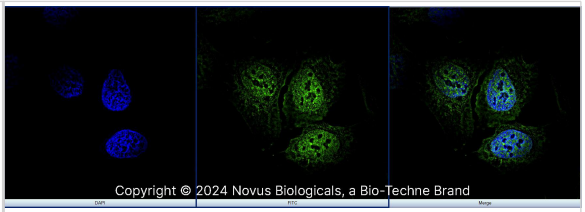
Immunohistochemistry: RBFOX3/NeuN Antibody [NBP1-77686] - Analysis of NeuN in mouse brain using DAB with hematoxylin counterstain.



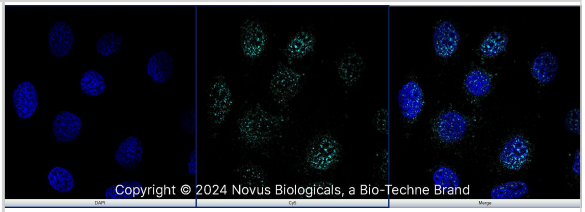
Flow Cytometry: RBFOX3/NeuN Antibody [NBP1-77686] - An intracellular stain was performed on U2OS cells with RBFOX3/NeuN Antibody NBP1-77686AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



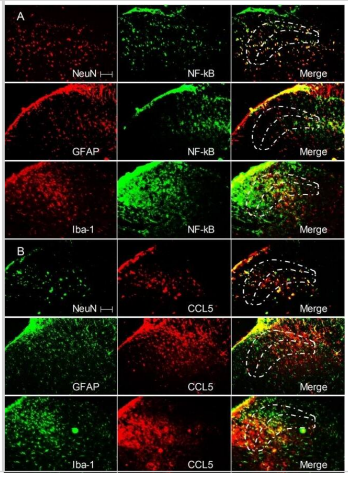
RBFOX3/NeuN was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti-RBFOX3/NeuN Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP1-77686AF488) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



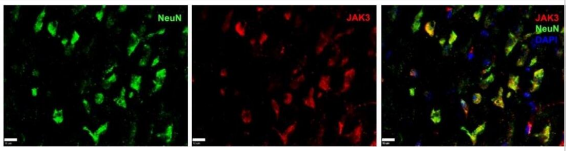
RBFOX3/NeuN was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti-RBFOX3/NeuN Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-77686AF647) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Immunocytochemistry/ Immunofluorescence: RBFOX3/NeuN Antibody - BSA Free [NBP1-77686] - Double immunofluorescence indicates that NF-κB & CCL5 were co-localized with microglia, astrocytes, & neurons in the ipsilateral L4–5 spinal cord on day 7 after CCI.NF-κB (green) colocalizes with Iba-1, GFAP, & NeuN (red) in laminae II–III of the superficial dorsal horn (A). CCL5 (red) co-localizes with Iba-1, GFAP, & NeuN (green) in the medial superficial dorsal horn (laminae II–III) (B). NF-κB (green) co-localizes with CCL5 (red) in laminae II–IV of the superficial dorsal horn following CCI on day 7 in the sham & CCI groups in the ipsilateral L4–5 spinal cord (C). Two single stained images (yellow) were merged. Scale = 100 µm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0115120), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: RBFOX3/NeuN Antibody - BSA Free [NBP1-77686] - (A) Representative Western blots depicting phosphorylated JAK3 (pJAK3), total JAK3, & corresponding β-actin levels in the ipsilateral (CXI) versus the contralateral (CXC) cortex at 24 h in sham & mice subjected to permanent middle cerebral artery occlusion (pMCAO). (B,C) Quantified pJAK3 & JAK3 values normalized to β-actin in the cortex of sham & mice subjected to stroke. Both pJAK3 & JAK3 are significantly increased in the ipsilateral cortex of stroked animals (*P < 0.05 & **P < 0.01 with respect to sham ipsilateral/contralateral & stroke contralateral; sham, n = 5; stroke, n = 13). Data were analyzed using a two-way ANOVA (ipsilateral/contralateral or sham/stroke) with Bonferroni post-test. (D) Diagram showing peri-infarct brain region from which images were taken (n = 3). Scale bar is 10 µm, & magnification is 60×. (E) JAK3 colocalized with microglia/macrophages labeled with Iba-1. (F) JAK3 colocalized with neurons labeled with NeuN. (G) JAK3 colocalized with endothelial cells labeled with CD31. (H) JAK3 is not colocalized with astrocytes labeled with GFAP. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28790974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

de Paiva I, Silva R, Mendonça I et al. Semaglutide Attenuates Anxious And Depressive-Like Behaviors and Reverses The Cognitive Impairment in a Type 2 Diabetes Mellitus Via The Microbiota-Gut-Brain Axis Research Square 2023-09-15 (IHC-P, Mouse)

Wang W, Cao Q, Tan T et al. Epigenetic treatment of behavioral and physiological deficits in a tauopathy mouse model Aging Cell 2021-10-01 [PMID: 34547169] (Immunohistochemistry)

Williams JB, Cao Q, Yan Z. Transcriptomic analysis of human brains with Alzheimer's disease reveals the altered expression of synaptic genes linked to cognitive deficits Brain Communications 2021-07-01 [PMID: 34423299] (Immunohistochemistry)

Issler O, van der Zee YY, Ramakrishnan A et al. The long noncoding RNA FEDORA is a cell type- and sex-specific regulator of depression Science Advances 2022-12-02 [PMID: 36449610] (Immunohistochemistry)

Li F, Liu Y, Li L et al. Brain-derived extracellular vesicles mediate traumatic brain injury associated multi-organ damage Biochemical and Biophysical Research Communications 2023-05-01 [PMID: 37163934] (Mouse)

Bogdanov L, Shishkova D, Mukhamadiyarov R et al. Excessive Adventitial and Perivascular Vascularisation Correlates with Vascular Inflammation and Intimal Hyperplasia International journal of molecular sciences 2022-10-12 [PMID: 36293013] (IF/IHC, Rat)

Details:
Dilution used in IHC 1:100

Wilson CS, Dohare P, Orbeta S et al. Late Adolescence Mortality in Mice with Brain-Specific Deletion of the Volume-Regulated Anion Channel Subunit LRRC8A FASEB J 2021-09-01 [PMID: 34469026]

Stewart A, Glaser E, Mott CA et al. Advanced Age and Neurotrauma Diminish Glutathione and Impair Antioxidant Defense after Spinal Cord Injury Journal of neurotrauma 2022-04-04 [PMID: 35373589] (IF/IHC, Mouse)

Williams JB Transcriptomic and Epigenomic Analysis Reveals Convergent Synaptic Deficits in Alzheimer's Disease Thesis 2022-01-01 (IF/IHC, Human)

MendonCa IP, Paiva IHR, Duarte-Silva EP et al. Metformin and fluoxetine improve depressive-like behavior in a murine model of Parkinsons disease through the modulation of neuroinflammation, neurogenesis and neuroplasticity International immunopharmacology 2022-01-01 [PMID: 34890997] (IF/IHC, Mouse)

Yang C, Lavayen BP, Liu L Et al. Neurovascular protection by adropin in experimental ischemic stroke through an endothelial nitric oxide synthase-dependent mechanism Redox biology 2021-11-22 [PMID: 34826783] (IF/IHC, Mouse)

Kopper TJ Myelin, cPLA2, and Azithromycin: Modulation of Macrophage Activation in Spinal Cord Injury Inflammation Thesis 2021-01-01 (IF/IHC)

More publications at http://www.novusbio.com/NBP1-77686

Procedures

Immunohistochemistry-Paraffin Embedded Sections protocol specific for RBFOX/Neun antibody (NBP1-77686)

Immunohistochemistry-Paraffin Embedded Sections
Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.
Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.
*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunocytochemistry/Immunofluorescence protocol for RBFOX3/NeuN Antibody (NBP1-77686)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.
1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NBP1-77686

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP1-77686AF647	RBFOX3/NeuN Antibody [Alexa Fluor® 647]

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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