abcam

Product datasheet

Anti-NeuN antibody [EPR12763] - Neuronal Marker ab177487

Recombinant RabMAb

*** * * * 74 Abreviews 695 References 26 Images

Overview

Product name Anti-NeuN antibody [EPR12763] - Neuronal Marker

Description Rabbit monoclonal [EPR12763] to NeuN - Neuronal Marker

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), IHC (PFA fixed), mIHC, IHC-P, WB, ICC/IF, IHC-Fr

Species reactivity Reacts with: Mouse, Rat, Sheep, Goat, Cat, Dog, Human, Zebrafish, Common marmoset

Predicted to work with: Pig, Cynomolgus monkey ^

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: Mouse brain, mouse cerebellum, rat cerebellum and human fetal brain tissue lysates. ICC/IF:

SH-SY-5Y and Mouse primary neuron cells. IHC-P: Human cerebellum, human gliocytoma tissue. mIHC: Human cerebellum tissue IHC-Fr: Mouse dentate gyrus tissue. Flow Cyt (intra): U-87 MG

cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this

update, please contact our Scientific Support team.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

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term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR12763

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab177487 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

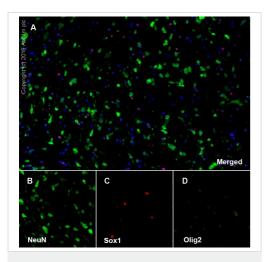
Application	Abreviews	Notes	
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.	
IHC (PFA fixed)		Use at an assay dependent concentration.	
mIHC		1/1000. Perform Sodium citrate antigen retrieval (pH 6.0) in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.	
IHC-P	★★★★★ (16)	1/3000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/800.	
WB	★★★★★ (6)	1/1000 - 1/10000. Detects a band of approximately 48,50 kDa (predicted molecular weight: 34 kDa). For unpurified use at 1/1000 - 1/2000.	
ICC/IF	★★★★ (16)	1/100 - 1/300. For unpurified use at 1/80.	
IHC-Fr	★★★★★ (17)	Use at an assay dependent concentration. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.	

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Function RNA-binding protein that regulates alternative splicing events.

Sequence similarities Contains 1 RRM (RNA recognition motif) domain.

Cellular localization Nucleus. Cytoplasm.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody
[EPR12763] - Neuronal Marker (ab177487)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse cerebrum tissue labelling NeuN with ab177487 at 1/100 dilution (B), SOX1 with ab242125 at 1/100 dilution (C) and Olig2 with ab109186 at 1/100 dilution (D). Anti-Rabbit and Mouse Polymer HRP was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins. Heat mediated antigen retrieval (Leica ER2, PH9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibodies from the previous round, to avoid any cross-reactivity.

Panel A: merged staining of anti- NeuN (green, Opal™520), anti-SOX1 (red, Opal™570) and anti- Olig2 (yellow, Opal™690).

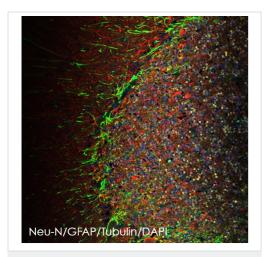
Panel B: anti-NeuN stained on neurons.

Panel C: anti-SOX1 stained on neural progenitors.

Panel D: anti-Olig2 stained on oligodendrocyte.

The section was incubated in three rounds of staining: in the order of ab177487, **ab242125** and **ab109186** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

Fluorescence multiplex immunohistochemical analysis of human cerebellum tissue (formalin-fixed paraffin-embedded section).

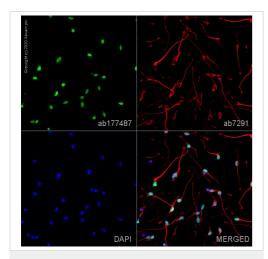
Merged staining of Neu-N (ab177487; yellow; OpalTM570), anti-beta III Tubulin (<u>ab52623</u>; red; OpalTM690) and anti-GFAP (<u>ab68428</u>; green; OpalTM520).

The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument with an Opal™ kit.

The section was incubated in three rounds of staining with ab177487 (1/1000 dilution), <u>ab52623</u> (1/200 dilution) and <u>ab68428</u> (1/250 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (pH 6.0) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

DAPI (blue) was used as a nuclear counter stain.



Immunocytochemistry/ Immunofluorescence - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

ab 177487

Immunocytochemistry/ Immunofluorescence - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

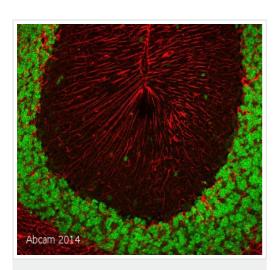
Immunofluorescence staining of NeuN using ab177487 in ioGlutamatergic Neurons (Human iPSC-Derived Glutamatergic Neurons, <u>ab259259</u>), which were differentiated for 1 day post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab177487 at 1 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with ab150081, Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150120, Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown.

Immunocytochemistry/immunofluorescence analysis of Mouse primary neuron cells labelling NeuN with ab177487 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 was used as the secondary antibody (green). Cells were counterstained with Anti-MAP2 mouse monoclonal antibody (ab11267) at 1/200 dilution and visualised using Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) at 1/1000 dilution (red). Nuclear DNA was labelled with DAPI (blue).

Confocal image showing mainly nuclear staining in mouse primary neuron cells. Confocal scanning Z step was set as $0.3 \mu m$ followed by image processing with maximum Z projection.

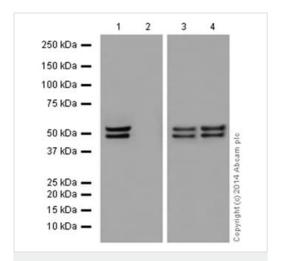


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody
[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

IHC-P image of NeuN (green) and GFAP (red) double staining on mouse cerebellum sections using ab177487 (1/5000) and **ab4674** (1/1500) respectively.

The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were then incubated with Rabbit Monoclonal to NeuN (ab177487) diluted at 1/5000 and Chicken Polyclonal to GFAP (ab4674) diluted at 1/1500. The primary antibody was detected using ab150097 Goat anti-rabbit IgG conjugated to Alexa Fluor® 488 (1/500) and ab150176 Goat anti-chicken IgY conjugated to Alexa Fluor® 594 (1/500)



Western blot - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

All lanes : Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487) at 1/10000 dilution (purified)

Lane 1: Human fetal brain tissue lysate

Lane 2: HEK-293 (Human epithelial cell line from embryonic

kidney) whole cell lysate

Lane 3: Mouse brain tissue lysate

Lane 4: Rat brain tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Peroxidase conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

Predicted band size: 34 kDa Observed band size: 46 kDa Exposure time -

Lane 1-2: 3 minutes.

Lane 3-4: 1 minute.

Blocking and dilution buffer: 5% NFDM/TBST.

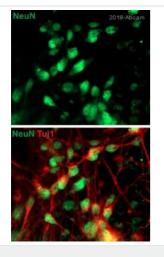
NeuN antibody used with Tissue Clearing Kit ab243298 on 1 mm mouse brain sections

Immunohistochemistry (PFA fixed) - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

NeuN antibody ab177487 was used with Tissue Clearing Kit ab243298 to penetrate, stain and clear a 1 mm coronal section of mouse brain. Blue: DAPI, Green: NeuN.

Learn more about <u>tissue clearing kits, reagents, and</u>
<u>protocols</u> designed to make it easier to stain thick tissue sections and get more data from each valuable tissue section.

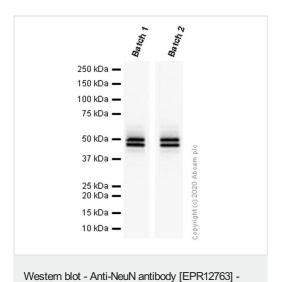
For 1 mm brain sections, we recommend a starting dilution of 1:200, and also using Goat Anti-Rabbit lgG H&L AlexaFluor488 (ab150077) at a dilution of 1:400.



Immunocytochemistry/ Immunofluorescence - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

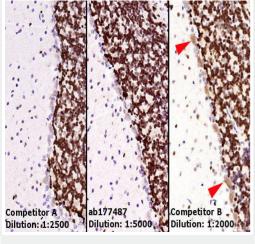
This image is courtesy of an Abreview submitted by Vaadimir Mlenkovic

Immunocytochemistry/immunofluorescence analysis of human neurons differentiated from iPSCs labelling NeuN (green) with ab177487 at 1/500 in 0.1% TritonX-100, 1% goat serum, 1X PBS for 16 hours at 4°C. Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100. Then, cells were blocked with 5% serum for 20 minutes at 23°C. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 was used as the secondary antibody. Tuj1 antibody was used to stain neuronal dendrites and axons (red).



Neuronal Marker (ab177487)

Different batches of ab177487 were tested on mouse brain lysate at 2.0 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 46,48 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffin-

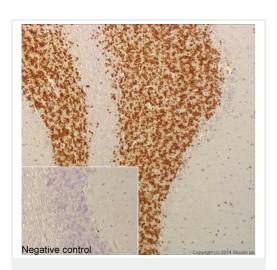
embedded sections) - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487) An independent comparison of commercially available NeuN clones in IHC-P.

Competitor A: Leading mouse monoclonal.

Competitor B: Non-Abcam rabbit monoclonal.

Sodium citrate was used for antigen retrieval in all 3 samples.

ab177487 produces specific staining, equivalent to the leading mouse monoclonal at half the dilution. The non-Abcam mouse monoclonal was less specific as it stained Purkinje cells, which do not express NeuN.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody

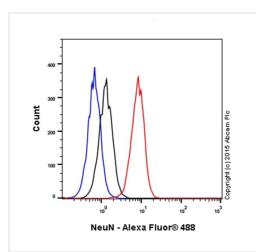
[EPR12763] - Neuronal Marker (ab177487)

IHC image of NeuN (ab177487) with Anti-Rabbit lgG VHH Single Domain Antibody (HRP) (ab191866) staining in formalin fixed paraffin embedded normal human cerebellum tissue section.

The section was dewaxed and then pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Dako Pascal pressure cooker using the standard factory-set regime. Non-specific protein-protein interactions were then blocked using in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 3% (w/v) BSA for 1 hour at room temperature. The section was then incubated with rabbit monoclonal antibody [EPR12763] to NeuN (ab177487, 0.1µg/ml) in TBS containing 0.025% (v/v) Triton X-100 and 3% (w/v) BSA overnight at +4°C. Endogenous peroxidases were quenched using 1.6% (v/v) hydrogen peroxide in TBS containing 0.025% (v/v) Triton X-100 for 30 minutes at room temperature, with agitation. The secondary antibody, Anti-Rabbit IgG VHH Single Domain Antibody (HRP) (ab191866, 1.0µg/ml) was then applied for 1 hour at room temperature in TBS containing 0.025% (v/v) Triton X-100 and 3% (w/v) BSA before being developed for 10 minutes at room temperature using Steady DAB/Plus (ab103723). The section was then counterstained with hematoxylin and mounted with DPX.

The negative control (secondary antibody only, no primary) inset shows no staining, demonstrating secondary antibody specificity.

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



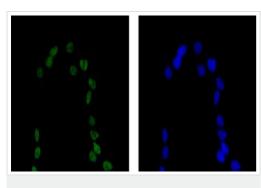
Flow Cytometry (Intracellular) - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

Overlay histogram showing U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) cells stained with ab177487 (red line).

The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab177487, 1/100 dilution) for 30 minutes at 22°C. The secondary antibody used was Alexa Fluor $^{\rm 8488}$ goat anti-rabbit lgG (H&L) (ab150081) at 1/2000 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (ab172730, 1µg/1x106 cells used under the same conditions. Unlabeled sample (blue line) was also used as a control.

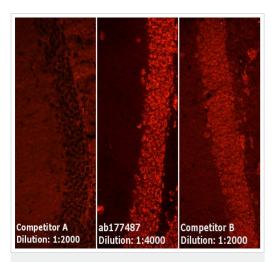
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Alexa Fluor[®] 488 (<u>ab190195</u>) and Alexa Fluor[®] 647 (<u>ab190565</u>) conjugated versions are available for this clone.



Immunocytochemistry/ Immunofluorescence - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

Immunocytochemsitry/Immunofluorescence analysis of SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells labeling NeuN (green) with ab177487 at 1/300. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).



Immunohistochemistry (Frozen sections) - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487) An independent comparison of commercially available NeuN clones in IHC-Fr (acetone-fixed mouse dentate gyrus sections).

Competitor A: Leading mouse monoclonal.

Competitor B: Non-Abcam rabbit monoclonal.

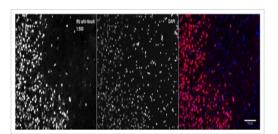
ab177487 produces intense, specific staining with minimal background, even at half the dilution of competing antibodies.



Immunohistochemistry (Frozen sections) - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an Abreview submitted by Jianning Lu

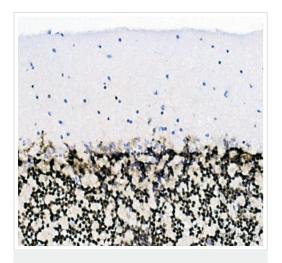
ab177487 staining NeuN in mouse free floating 50 micron lumbar spinal cord tissue sections by Immunohistochemistry (IHC-Fr-frozen sections). Tissue was fixed with formaldehyde, permeabilized with Triton X-100 and blocked with 10% serum for 2 hours at 25°C. Samples were incubated with primary antibody (1/500 in PBS + Triton) for 16 hours at 4°C. An Alexa Fluor[®] 594-conjugated donkey anti-rabbit IgG polyclonal (1/700) was used as the secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an Abreview submitted by Eva Borger

ab177487 staining NeuN in mouse brain tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with formaldehyde and blocked with Triton X-100 + 0.4% horse seurm for 30 minutes at 20°C. Samples were incubated with primary antibody (1/500 in blocking solution) for 16 hours at 4°C. An Alexa Fluor[®] 594-conjugated donkey anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.

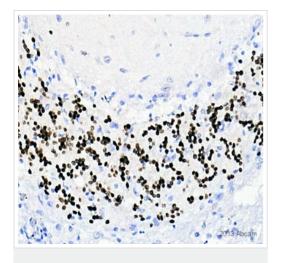


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody
[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

IHC-P image of FOX3/NeuN staining on cat cerebellum sections using ab177487 (1/1000).

Sections were de-paraffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/1000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit lgG conjugated to biotin (1/250).



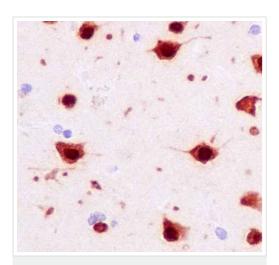
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody

[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

IHC-P image of FOX3/NeuN staining on dog cerebellum sections using ab177487 (1/500).

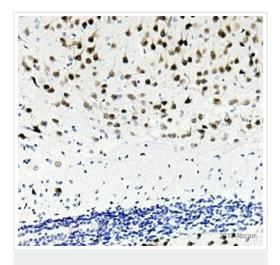
Sections were de-paraffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/500 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit lgG conjugated to biotin (1/250).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody

[EPR12763] - Neuronal Marker (ab177487)

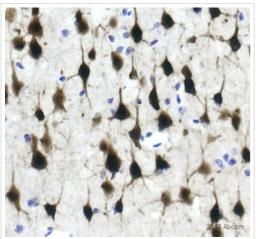
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gliocytoma tissue labelling NeuN with ab177487 at 1/3000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Counterstained with Hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody
[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

IHC-P image of FOX3/NeuN staining on rat brain (SVZ) sections using ab177487 (1/2000). Sections were de-paraffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/2000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to biotin (1/250).

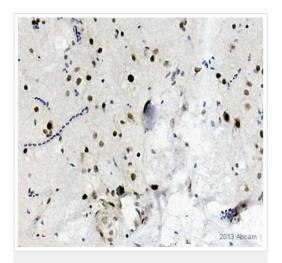


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

[EPR12763] - Neuronal Marker (ab177487)

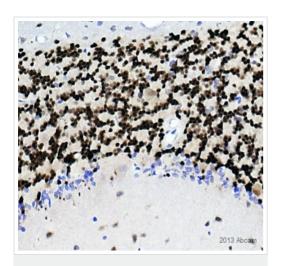
IHC-P image of FOX3/NeuN staining on mouse brain (frontal cortex) sections using ab177487 (1/800). Sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/800 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to biotin (1/250).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody
[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

IHC-P image of FOX3/NeuN staining on zebrafish spinal cord sections using ab177487 (1/500). Sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/500 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to biotin (1/250).

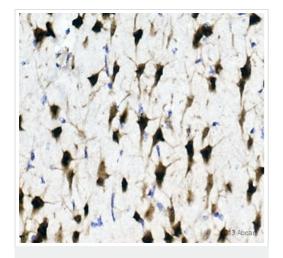


IHC-P image of FOX3/NeuN staining on marmoset cerebellum sections using ab177487 (1/2000). Sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/2000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to biotin (1/250).

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody

[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody
[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.

IHC-P image of FOX3/NeuN staining on sheep brain (Frontal cortex) sections using ab177487 (1/1000). Sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/1000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to biotin (1/250).



IHC-P image of FOX3/NeuN staining on goat cerebellum sections using ab177487 (1/500). Sections were de-paraffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 minutes at 21°C. ab177487 was diluted 1/500 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit lgG conjugated to biotin (1/250).

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NeuN antibody

[EPR12763] - Neuronal Marker (ab177487)

This image is courtesy of an abreview submitted by Carl Hobbs, Kings's College London, United Kingdom.



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