Anti-NeuN Antibody [SR45-07]

ET1602-12

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IHC-Fr, mIHC
Molecular Wt:	Predicted band size: 34 kDa
Clone number:	SR45-07

Description: Neuronal nuclei (NeuN, Fox-3, RBFOX3) is a nuclear protein expressed in most post-mitotic neurons of the central and peripheral nervous systems. NeuN is not detected in Purkinje cells, sympathetic ganglion cells, Cajal-Retzius cells, INL retinal cells, inferior olivary, and dentate nucleus neurons. This neuronal protein was originally identified by immunoreactivity with a monoclonal antibody also called NeuN. Using MS-analysis, NeuN was later identified as the Fox-3 gene product. Fox-3 contains an RNA recognition motif and functions as a splicing regulator. Fox-3 regulates alternative splicing of NumB, promoting neuronal differentiation during development.

Immunogen: Synthetic peptide within human NeuN aa 20-60.

Positive control: Mouse brain tissue lysate, rat brain tissue lysate, mouse cerebellum tissue lysate, rat cerebellum tissue lysate, SH-SY5Y cell lysate, SHG-44 cell lysate, rat cerebellum tissue, primary mouse neurons/glia cells, mouse cerebral cortex tissue, mouse cerebellum tissue, rat cerebral cortex tissue, mouse hippocampus tissue, rat hippocampus tissue, human brain tissue, human cerebellum tissue, human glioblastoma tissue, SH-SY5Y, mouse brain tissue.

Subcellular location: Cytoplasm, Nucleus

Database links: SwissProt: A6NFN3 Human | Q8BIF2 Mouse
Unigene: 143966 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:250-1:500
IF-Tissue	1:200-1:1,000
IHC-P	1:200-1:10,000
IHC-Fr	1:50-1:100
FC	1:1,000
mIHC	1:5,000-1:10,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

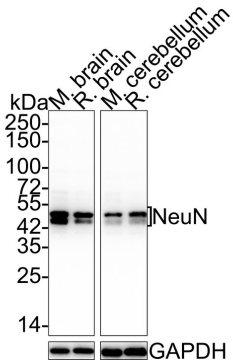

Fig1: Western blot analysis of NeuN on different lysates with Rabbit anti-NeuN antibody (ET1602-12) at 1/5,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Rat brain tissue lysate
Lane 3: Mouse cerebellum tissue lysate
Lane 4: Rat cerebellum tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 45/50 kDa

Exposure time: 43 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1602-12) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

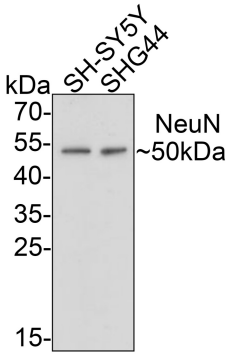

Fig2: Western blot analysis of NeuN on different lysates with Rabbit anti-NeuN antibody (ET1602-12) at 1/500 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: SHG-44 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 50 kDa

Exposure time: 2 minutes;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1602-12) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

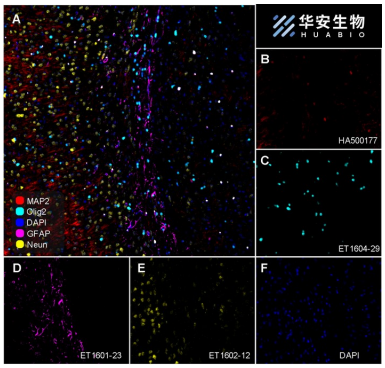

Fig3: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-MAP2 (HA500177, Red), anti-Olig2 (ET1604-29, Cyan), anti-GFAP (ET1601-23, Magenta) and anti-Neun (ET1602-12, Yellow) on mouse brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA500177 (1/1,000 dilution), ET1604-29 (1/5,000 dilution), ET1601-23 (1/10,000 dilution) and ET1602-12 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

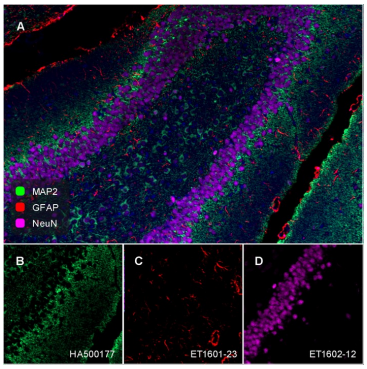

Fig4: Fluorescence multiplex immunohistochemical analysis of mouse hippocampus (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-MAP2 (HA500177, Green), anti-GFAP (ET1601-23, Red) and anti-NeuN (ET1602-12, Magenta) on Mouse hippocampus. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of HA500177 (1/1,000 dilution), ET1601-23 (1/1,000 dilution) and ET1602-12 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

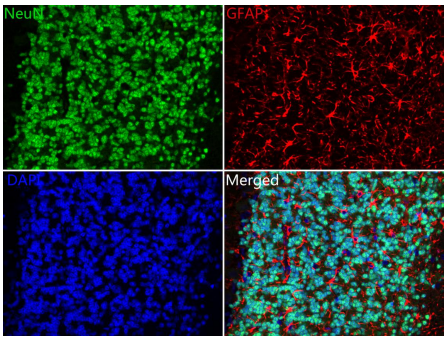

Fig5: Immunofluorescence analysis of paraffin-embedded rat cerebellum tissue labeling NeuN (ET1602-12) and GFAP (EM140707).

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies NeuN (ET1602-12, Green) at 1/50 dilution and GFAP (EM140707, Red) at 1/500 dilution overnight at 4 ℃, washed with PBS. iFluor™ 488 conjugate-Goat anti-Rabbit IgG (HA1121) and iFluor™ 594 conjugate-Goat anti-Mouse IgG (HA1126) were used as the secondary antibody at 1/1,000 dilution. DAPI was used as nuclear counterstain.

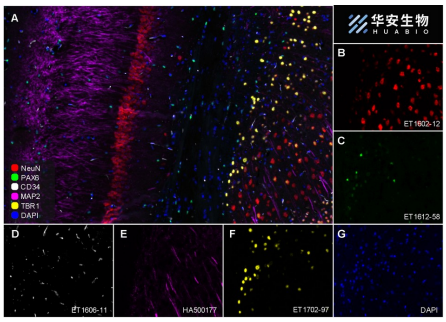

Fig6: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-PAX6 (ET1612-58, green), anti-CD34 (ET1606-11, gray), anti-MAP2 (HA500177, magenta) and anti-TBR1 (ET1702-97, yellow) on mouse brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of ET1602-12 (1/5,000 dilution), ET1612-58 (1/1,000 dilution), ET1606-11 (1/2,000 dilution), HA500177 (1/5,000 dilution) and ET1702-97 (1/1,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

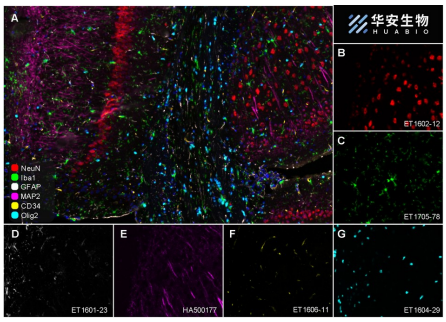

Fig7: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-Iba1 (ET1705-78, green), anti-GFAP (ET1601-23, gray), anti-Olig2 (ET1604-29, cyan), anti-MAP2 (HA500177, magenta) and anti-CD34 (ET1606-11, yellow) on mouse brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of ET1602-12(1/5,000 dilution), ET1705-78 (1/2,000 dilution), ET1601-23 (1/5,000 dilution), ET1604-29 (1/1,000 dilution), HA500177 (1/5,000 dilution) and ET1606-11 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

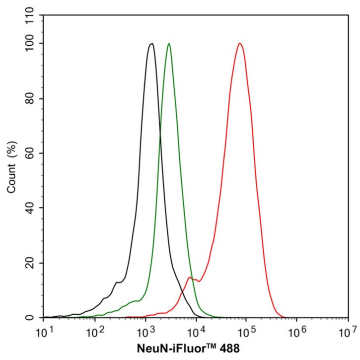

Fig8: Flow cytometric analysis of SHG-44 cells labeling NeuN.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1602-12, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

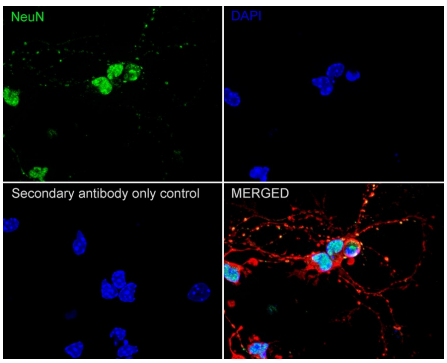

Fig9: Immunocytochemistry analysis of primary mouse neurons/glia cells labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12) at 1/500 dilution.

Cells were fixed with 4% PFA (15 min), permeabilized with 0.25% TritonX-100 for 15 minutes 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℃ with Rabbit anti-NeuN antibody (ET1602-12) at at 1/500 dilution. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

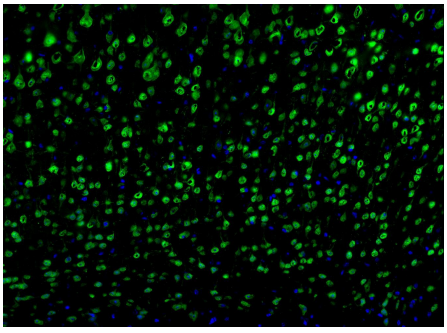

Fig10: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1602-12, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

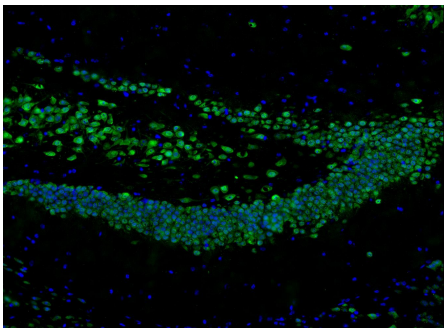

Fig11: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1602-12, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

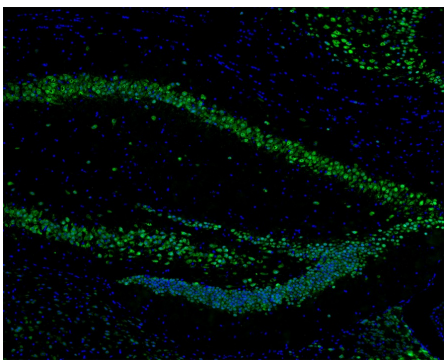

Fig12: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1602-12, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

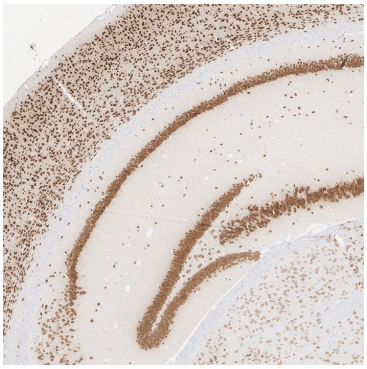

Fig13: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

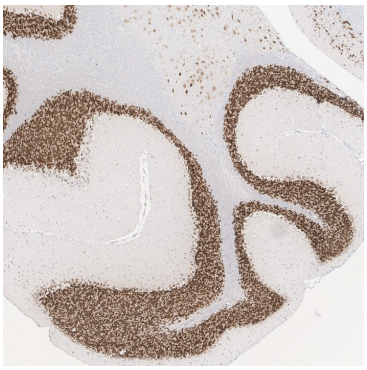

Fig14: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

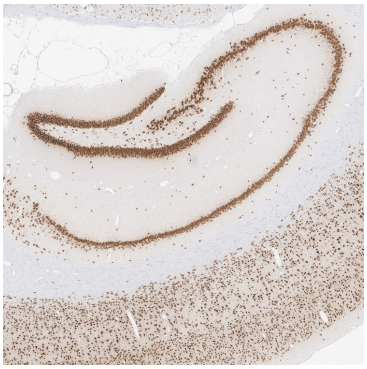

Fig15: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

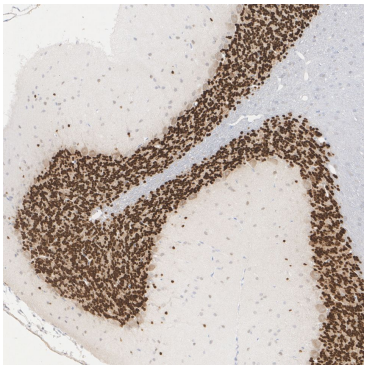

Fig16: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

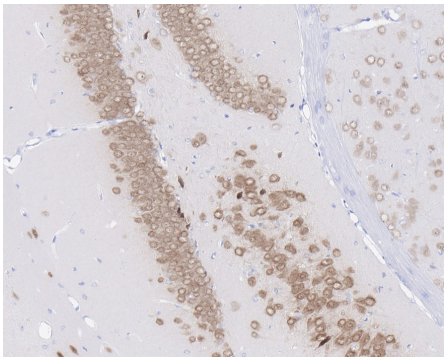

Fig17: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

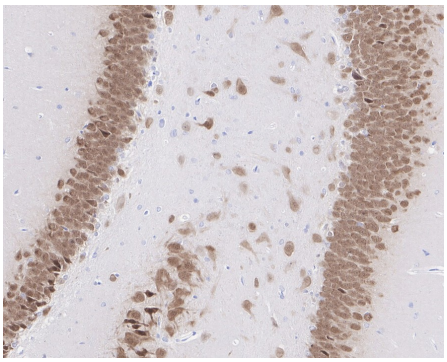

Fig18: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

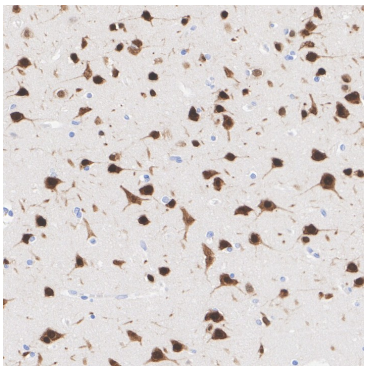

Fig19: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

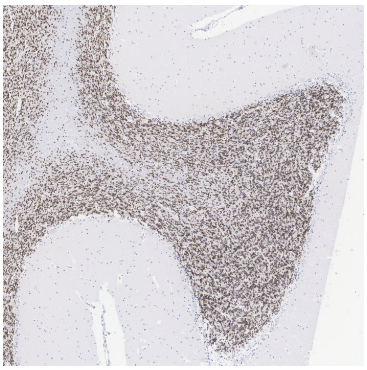

Fig20: Immunohistochemical analysis of paraffin-embedded human cerebellum tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

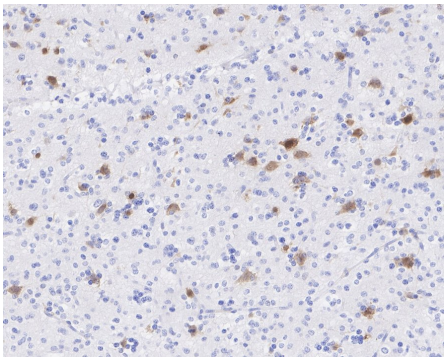

Fig21: Immunohistochemical analysis of paraffin-embedded human glioblastoma tissue with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

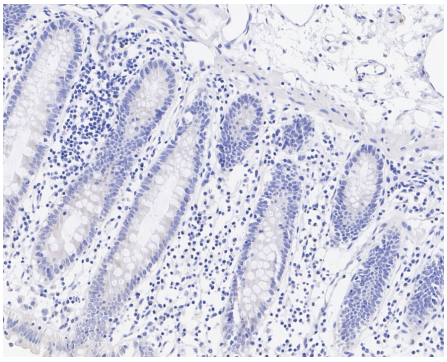

Fig22: Immunohistochemical analysis of paraffin-embedded human colon tissue (negative) with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-12) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

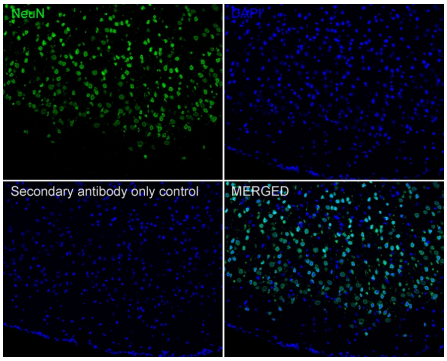

Fig23: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1602-12, green) at 1/1,000 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

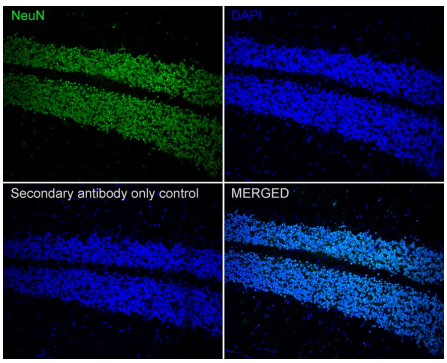

Fig24: Immunofluorescence analysis of paraffin-embedded mouse cerebellum tissue labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1602-12, green) at 1/1,000 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

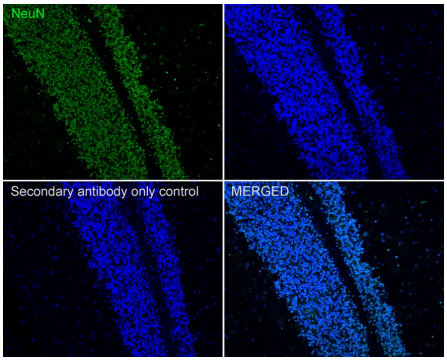

Fig25: Immunofluorescence analysis of paraffin-embedded rat cerebellum tissue labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1602-12, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

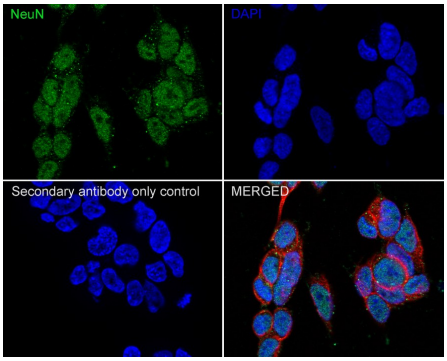

Fig26: Immunocytochemistry analysis of SH-SY5Y cells labeling NeuN with Rabbit anti-NeuN antibody (ET1602-12) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NeuN antibody (ET1602-12) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Patel TP et al. Single-neuron NMDA receptor phenotype influences neuronal rewiring and reintegration following traumatic injury. J Neurosci 34:4200-13 (2014).
2. Kaur P et al. Expression profiling of RNA transcripts during neuronal maturation and ischemic injury. PLoS One 9:e103525 (2014).