Anti-NF-L Antibody [PSH0-77] - BSA and Azide free

HA750657

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue
Molecular Wt:	Predicted band size: 62 kDa
Clone number:	PSH0-77

Description: Neurofilament light polypeptide, also known as neurofilament light chain, abbreviated to NF-L or Nfl and with the HGNC name NEFL is a member of the intermediate filament protein family. This protein family consists of over 50 human proteins divided into 5 major classes, the Class I and II keratins, Class III vimentin, GFAP, desmin and the others, the Class IV neurofilaments and the Class V nuclear lamins. There are four major neurofilament subunits, NF-L, NF-M, NF-H and α-internexin. These form heteropolymers which assemble to produce 10nm neurofilaments which are only expressed in neurons where they are major structural proteins, particularly concentrated in large projection axons. Axons are particularly sensitive to mechanical and metabolic compromise and as a result axonal degeneration is a significant problem in many neurological disorders. The detection of neurofilament subunits in CSF and blood has therefore become widely used as a biomarker of ongoing axonal compromise. The NF-L protein is encoded by the NEFL gene. Neurofilament light chain is a biomarker that can be measured with immunoassays in cerebrospinal fluid and plasma and reflects axonal damage in a wide variety of neurological disorders. It is a useful marker for disease monitoring in amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, and more recently Huntington's disease. It is also promising marker for follow-up of patients with brain tumors. Higher levels of blood or CSF NF-L have been associated with increased mortality, as would be expected as release of this protein reflects ongoing axonal loss. Recent work performed as a collaboration between EnCor Biotechnology Inc. and the University of Florida showed that the NF-L antibodies employed in the most widely used NF-L assays are specific for cleaved forms of NF-L generated by proteolysis induced by cell death.

Immunogen: Recombinant protein.

Positive control: Mouse brain tissue lysate, rat brain tissue lysate, mouse hippocampus tissue lysate, rat hippocampus tissue lysate, human brain tissue, mouse brain tissue, rat cerebellum tissue, SH-SY5Y.

Subcellular location: Cell projection, axon, Cytoplasm, cytoskeleton.

Database links: SwissProt: P07196 Human | P08551 Mouse | P19527 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:2,000
IF-Cell	1:200
IF-Tissue	1:200

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

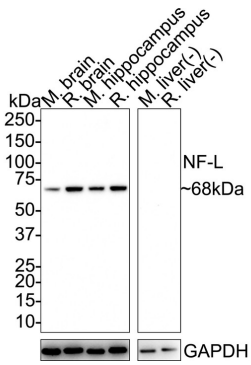

Fig1: Western blot analysis of NF-L on different lysates with Rabbit anti-NF-L antibody (HA750657) at 1/1,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Rat brain tissue lysate
Lane 3: Mouse hippocampus tissue lysate
Lane 4: Rat hippocampus tissue lysate
Lane 5: Mouse liver tissue lysate (negative)
Lane 6: Rat liver tissue lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 62 kDa
Observed band size: 68 kDa

Exposure time: 3 minutes;

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 (HA750657) at 1/1,000 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.

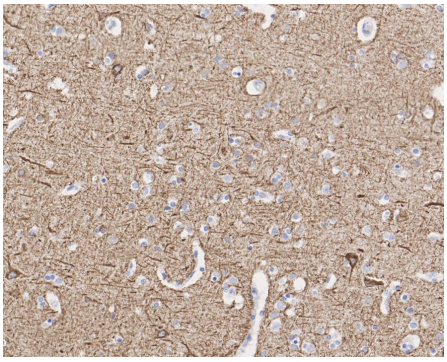

Fig2: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-NF-L antibody (HA750657) at 1/2,000 dilution.

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 (HA750657) at 1/2,000 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.

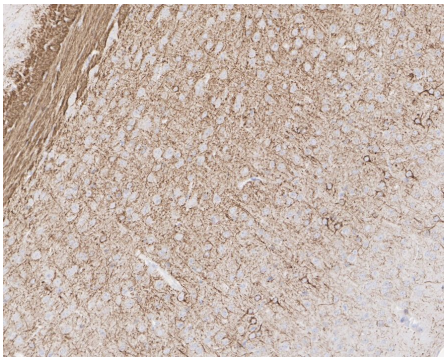

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-NF-L antibody (HA750657) at 1/2,000 dilution.

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 (HA750657) at 1/2,000 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.

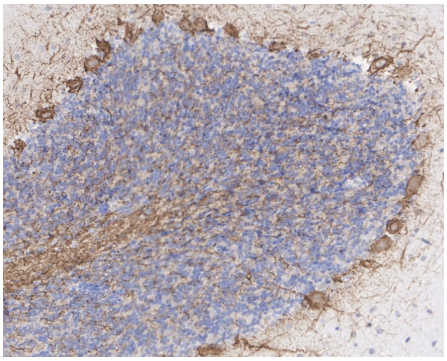

Fig4: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-NF-L antibody (HA750657) at 1/2,000 dilution.

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 (HA750657) at 1/2,000 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.

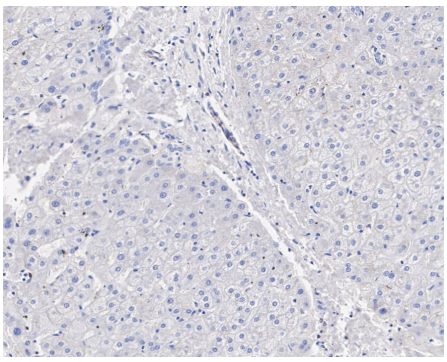

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue (negative) with Rabbit anti-NF-L antibody (HA750657) at 1/2,000 dilution.

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 (HA750657) at 1/2,000 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.

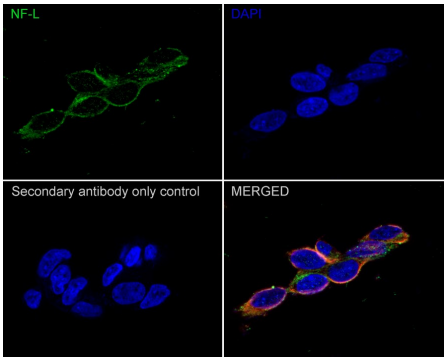

Fig6: Immunocytochemistry analysis of SH-SY5Y cells labeling NF-L with Rabbit anti-NF-L antibody (HA750657) at 1/200 dilution.

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-NF-L antibody (HA750657) at 1/200 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.

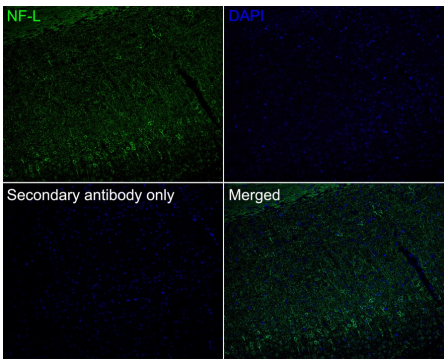

Fig7: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling NF-L with Rabbit anti-NF-L antibody (HA750657) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750657, green) at 1/200 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).

Background References

1. Gong L et al. Neurofilament Light Chain (NF-L) Stimulates Lipid Peroxidation to Neuronal Membrane through Microglia-Derived Ferritin Heavy Chain (FTH) Secretion. Oxid Med Cell Longev. 2022 Mar
2. Heiskanen M et al. Plasma Neurofilament Light Chain (NF-L) Is a Prognostic Biomarker for Cortical Damage Evolution but Not for Cognitive Impairment or Epileptogenesis Following Experimental TBI. Int J Mol Sci. 2022 Dec