HRP anti-Neurofilament H (NF-H), Phosphorylated Antibody

Catalog# / Size	801605 / 25 µg 801606 / 100 µg
Clone	SMI 31
Regulatory Status	RUO
Other Names	Neurofilament heavy polypeptide, NF-H, 200 kD neurofilament protein, neurofilament triplet H protein
Isotype	Mouse IgG1, κ
Description	Neurofilaments (NF) are approximately 10 nanometer intermediate filaments found in neurons. They are a major component of the neuronal cytoskeleton, and function primarily to provide structural support for the axon and to regulate the axon diameter. There are three major NF subunits, and the names given to these subunits are based upon the apparent molecular mass of the mammalian subunits on SDS-PAGE. The light or lowest NF (NF-L) runs at 68-70 kD. The medium or middle NF (NF-M) runs at about 145-160 kD, and the heavy or highest NF (NF-H) runs at 200-220 kD. However, the actual molecular weight of these proteins is considerably lower due to the highly charged C-terminal regions of the molecules. The level of NF gene expression correlates with the axonal diameter, which controls how fast electrical signals travel down the axon. Mutant mice with NF abnormalities have phenotypes resembling amyotrophic lateral sclerosis. NF immunostaining is common in diagnostic neuropathology. It is useful for differentiating neurons (positive for NF) from the glia (negative for NF).

Product Details

Verified Reactivity	Human, Mouse, Rat
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	This antibody is provided in 50% glycerol in aqueous buffered solutions with preservatives.
Preparation	The antibody was purified by affinity chromatography and conjugated with HRP under optimal conditions.
Concentration	0.5 mg/ml
Storage & Handling	Upon receipt, the antibody solution should be stored undiluted at -20°C, and protected from prolonged exposure to light.
Application	IHC-P - *Quality tested* WB - *Verified*
Recommended Usage	Each lot of this antibody is quality control tested by formalin-fixed paraffin-embedded immunohistochemical staining. For immunohistochemisty, a concentration range of 1.0 - 5.0 µg/ml is suggested. For Western blotting, the suggested use of this reagent is 2.5 - 5.0 µg per ml. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	Additional reported applications (for the relevant formats) include: Western blotting[1], immunohistochemistry[2,4], and immunocytochemistry[4]. SMI 31 reacts with a phosphorylated epitope in extensively phosphorylated neurofilament H and, to a lesser extent, with neurofilament M in most mammalian species, which chicken and frog (Xenopus). Immunocytochemically, SMI 31 reacts broadly with thick and thin axons and some dendrites such as basket cell dendrites, but not Purkinje cell dendrites. Nerve cell bodies are generally unreactive. Other cells and tissues are unreactive except for peripheral axons. Phosphatase treatment of tissue sections or Western blots abolishes reaction with SMI 31. Staining is unaffected by trypsin. In pathological conditions, reaction with SMI 31 may be found also in neuronal cell bodies. Aberrant phosphorylation of neurofilament H in cell bodies can be demonstrated in neuronal cell cultures with SMI 31 by agents that induce stress-activated protein kinase. In its reaction with paired helical filaments in hereditary inclusion body myopathy, SMI 31 colocalizes with nitric oxide synthase, suggesting that oxidative stress may play a role in the pathogenic cascade of such degenerative diseases. SMI 31 co-immunoprecipitates neurofilament-

associated kinase (NAK 115) via reaction of the antibody with the tail domain of neurofilament H.

Application References

(PubMed link indicates BioLegend citation)

1. Barry D, et al. 2012. *J. Neurosci.* 32:6209 **(WB)** PubMed
2. Choi Y, et al. 2008. *Genes Dev.* 22:2485. **(IHC)** PubMed
3. Sepulveda B, *et al.* 2013. *PLoS ONE.* 8(e61986. **(ICC)** PubMed
4. McLean NA, *et. al.* 2014. *PLoS One* 9:e110174. **(IHC)** PubMed

RRID

AB_2728522 (BioLegend Cat. No. 801605)
AB_2728523 (BioLegend Cat. No. 801606)

Antigen Details

Structure	Neurofilament H has an apparent molecular mass of 200-220 kD.
Distribution	Tissue distribution: CNS, peripheral nerves and glandular cells of the prostate. Cellular distribution: Cytoskeleton, nucleus, cytosol, and mitochondrion.
Function	Neurofilaments are the major components of the neuronal cytoskeleton. They provide axonal support and regulate axon diameter.
Interaction	Cell bodies and dendrites are generally unstained. Other cells and tissues are unreactive except for peripheral axons.
Cell Type	Mature Neurons
Biology Area	Cell Biology, Neuroscience, Neuroscience Cell Markers
Molecular Family	Intermediate Filaments, Phospho-Proteins
Antigen References	1. Petzold A. 2005. *J. Neurol. Sci.* 233 (1-2):183. PubMed
Gene ID	4744

Related Protocols

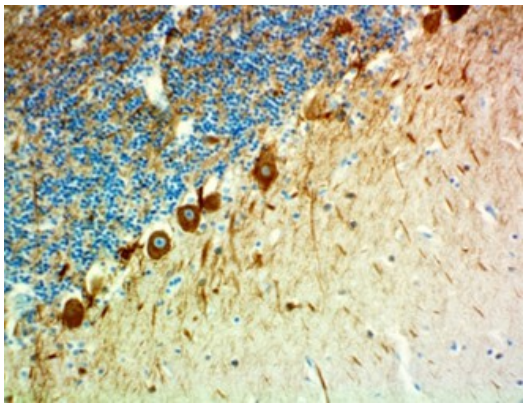
Western Blotting Protocol

Immunohistochemistry Protocol for Paraffin-Embedded Sections

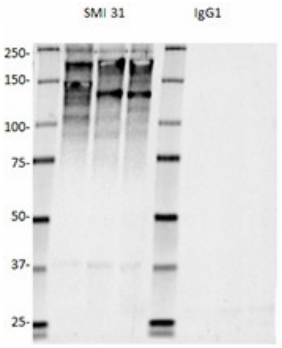
Other Formats

Purified anti-Neurofilament H (NF-H), Phosphorylated, Biotin anti-Neurofilament H (NF-H), Phosphorylated, Alexa Fluor® 594 anti-Neurofilament H (NF-H), Phosphorylated, HRP anti-Neurofilament H (NF-H), Phosphorylated, Alexa Fluor® 488 anti-Neurofilament H (NF-H), Phosphorylated, Alexa Fluor® 647 anti-Neurofilament H (NF-H), Phosphorylated

Product Data



IHC staining of HRP anti-Neurofilament H (NF-H), Phosphorylated Antibody (clone SMI 31) on formalin-fixed paraffin-embedded human cerebellum tissue. After antigen retrieval using Retrieve-All Antigen Unmasking System 3 (Cat. No. 927801), the tissue was incubated with the primary antibody at 5.0 µg/mL for one hour at room temperature. DAB was used for detection followed by hematoxylin counterstaining and bluing solution counterstaining, according to the protocol provided.



Western blot of HRP Anti-Neurofilament H (NF-H), Phosphorylated Antibody (clone SMI 31) and Isotype-matched IgG1 control. Lane 1: 20 µg of human brain lysate; Lane 2: 20 µg of rat brain lysate; Lane 3: 20 µg of mouse brain lysate. The blots were incubated with 1.0 µg/mL SMI 31 or mouse IgG1, κ overnight at 4°C. HRP NF-H was visualized using chemiluminescence detection. HRP labeled goat anti-mouse secondary antibody was used for the blot that was incubated with the IgG1 control antibody followed by chemiluminescence detection.

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