Purified anti-Neurofilament H & M (NF-H/NF-M), Phosphorylated Antibody

Catalog# / Size	837703 / 25 µg 837704 / 100 µg
Clone	SMI 310
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 regulate 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-L) runs at 68-70 kD; the medium or middle (NF-M) runs at about 145-160 kD; the heavy or highest (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 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 glia (negative for NF).

Product Details

Verified Reactivity	Human, Mouse, Rat
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.5 mg/ml
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C.
Application	IHC-P - *Quality tested* WB - *Verified* IHC-F, ICC - *Reported in the literature, not verified in house*
Recommended Usage	For immunohistochemistry, a concentration range of 0.5 - 10 µg/ml is suggested. For Western blotting, the suggested use of this reagent is 0.5 - 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[4], immunocytochemistry[2], immunohistochemical staining of frozen tissue sections[1,3], and spatial biology (IBEX)[5,6]. Clone SMI 310 reacts with an extensively phosphorylated epitope of neurofilament H and, to a much lesser extent, neurofilament M in most mammalian species. Phosphatase treatment of samples abolishes reaction with SMI 310. A very extensive degree of hyperphosphorylation of neurofilaments seems to be necessary for its reactivity.

Application References

(PubMed link indicates BioLegend citation)

1. Dubourg O, *et al.* 2011. *Acta Myol.* 30(2):103. (IHC-F) PubMed
2. Mizui T, *et al.* 2009. *J Neurochem.* 109(2):611. (ICC)
3. Denlger-Crish C, *et al.* 2014. *Front Neursci.* 8:290. (IHC-F) PubMed
4. Mulot SF, *et al.* 1994. *FEBS Lett.* 349(3):359. (WB)
5. Radtke AJ, *et al.* 2020. *Proc Natl Acad Sci U S A.* 117:33455-65. (SB) PubMed
6. Radtke AJ, *et al.* 2022. *Nat Protoc.* 17:378-401. (SB) PubMed

RRID	AB_2715865 (BioLegend Cat. No. 837703) AB_2566638 (BioLegend Cat. No. 837704)

Antigen Details

Structure	The medium or middle neurofilament (NF-M) runs at 145-160 kD, and the heavy or highest neurofilament (NF-H) runs at 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 while 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. Siedler D, *et al*. 2014. *Front Cell Neurosci*. 8:429.
Gene ID	4744

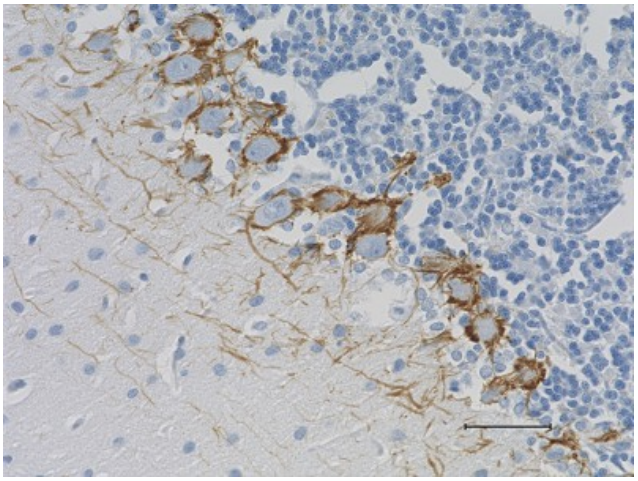
Related Protocols

Immunohistochemistry Protocol for Sternberger Monoclonal Antibodies

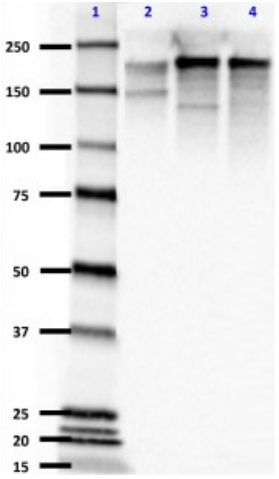
Other Formats

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

Product Data



IHC staining of purified anti-Neurofilament H & M (NF-H/NF-M), Phosphorylated antibody (clone SMI 310) on formalin-fixed paraffin-embedded rat brain tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3: Acidic, 100X (Cat. No. 927601), the tissue was incubated with 0.5 µg/ml of the primary antibody for 60 minutes at room temperature. BioLegend's Ultra-Streptavidin (USA) HRP kit (Multi-Species, DAB, Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. The image was captured with a 40X objective. Scale bar: 50 µm



Western blot of purified anti-Neurofilament H & M (NF-H/NF-M), Phosphorylated antibody (clone SMI 310). Lane 1: Molecular weight marker; Lane 2: 20 µg of human brain lysate; Lane 3: 20 µg of mouse brain lysate; Lane 4: 20 µg of rat brain lysate. The blot was incubated with 5 µg/mL of the primary antibody overnight at 4°C, followed by incubation with HRP labeled goat anti-mouse IgG (Cat. No. 405306). Enhanced chemiluminescence was used as the detection system.

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