

Alexa Fluor® 647 anti NF-H/NF-M, Hypophosphorylated Antibody

Catalog# / Size	835611 / 25 µg 835612 / 100 µg
Clone	SMI 35
Regulatory Status	RUO
Other Names	Neurofilament heavy polypeptide, NF-H, 200 kD neurofilament protein, neurofilament triplet H protein
Isotype	Mouse IgG1, κ
Description	Neurofilaments (NFs) 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 neurofilament (NF-L) runs at 68-70 kD. The medium or middle (NF-M) runs at about 145-160 kD, and 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 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	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 647 under optimal conditions.
Concentration	0.5 mg/ml
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. Do not freeze.
Application	IHC-P - Quality tested
Recommended Usage	<p>Each lot of this antibody is quality control tested by formalin-fixed paraffin-embedded immunohistochemical staining. For immunohistochemistry, a concentration range of 5 - 10 µg/ml is suggested. It is recommended that the reagent be titrated for optimal performance for each application.</p> <p>* Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633 nm / 635 nm.</p> <p>Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation.</p> <p>View full statement regarding label licenses</p>
Excitation Laser	Red Laser (633 nm)
Application Notes	<p>Additional reported applications (for the relevant formats of this clone): include ELISA Capture¹⁻³, immunohistochemical staining on frozen tissue sections, immunofluorescence staining, and spatial biology (IBEX)^{6,7}.</p> <p>Clone SMI 35 reacts with highly phosphorylated neurofilaments, as well as with low degrees of phosphorylation. It primarily reacts with neurofilament H and with neurofilament M to a lesser extent.</p> <p>Notes: On two dimensional gels, this antibody detects a band extending from the phosphorylated</p>

neurofilament position at 200 kD (pI 5.1) toward the non-phosphorylated position at 170 kD (pI 6.2).

Application References

(PubMed link indicates BioLegend citation)

1. Petzold A. 2013. *J. Neuroimmunol.* 262:(1-10). (ELISA)
2. Lu CH, et al. 2012. *PLoS One.* 7:e40998. (ELISA)
3. Steinacker P, et al. 2011. *PLoS One.* 8:e23600. (ELISA)
4. Poltorak M, et al. 1993. *J. Neurosci.* 13:2217. (IHC-F)
5. Petzold A, et al. 2011. *Brain.* 134:464. (WB) [PubMed](#)
6. Radtke AJ, et al. 2020. *Proc Natl Acad Sci U S A.* 117:33455-65. (SB) [PubMed](#)
7. Radtke AJ, et al. 2022. *Nat Protoc.* 17:378-401. (SB) [PubMed](#)

RRID

AB_2734602 (BioLegend Cat. No. 835611)
AB_2734603 (BioLegend Cat. No. 835612)

Antigen Details

Structure	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.
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	<ol style="list-style-type: none">1. Petzold A. 2012. <i>Mult Scler Int.</i> 2012:217802. PubMed2. Gresle MM, et al. 2011. <i>Mult Scler Int.</i> 2011: 315406. PubMed
Gene ID	4744

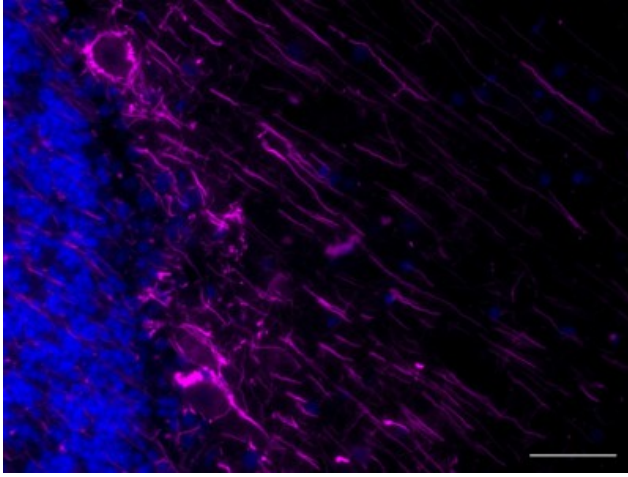
Related Protocols

[Immunohistochemistry Protocol for Paraffin-Embedded Sections](#)

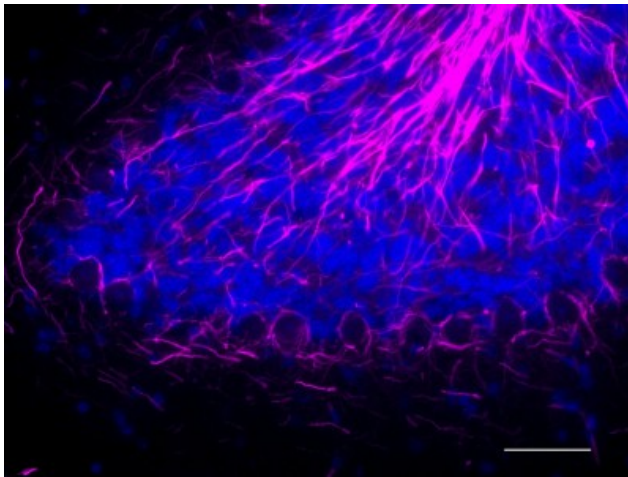
Other Formats

Anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated, Purified anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated, Biotin anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated, Alexa Fluor® 594 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated, HRP anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated, Alexa Fluor® 647 anti NF-H/NF-M, Hypophosphorylated, Alexa Fluor® 488 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated

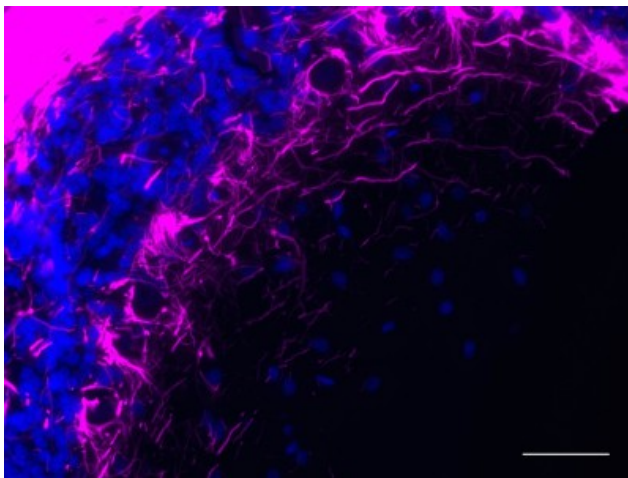
Product Data



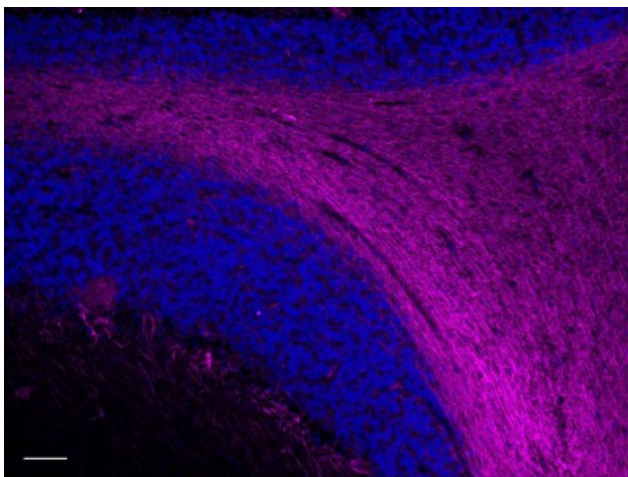
IHC staining of Alexa Fluor® 647 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated antibody (clone SMI 35) on formalin-fixed paraffin-embedded human brain tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3 (Cat. No. 927601), the tissue was incubated with 10 µg/ml of the primary antibody overnight at 4°C. The image was captured with a 40X objective. Scale bar: 50 µm



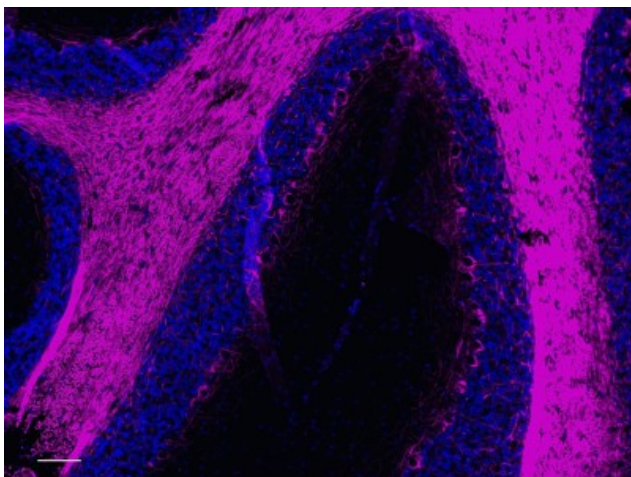
IHC staining of Alexa Fluor® 647 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated antibody (clone SMI 35) on formalin-fixed paraffin-embedded mouse brain tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3 (Cat. No. 927601), the tissue was incubated with 5 µg/ml of the primary antibody overnight at 4°C. The image was captured with a 40X objective. Scale bar: 50 µm



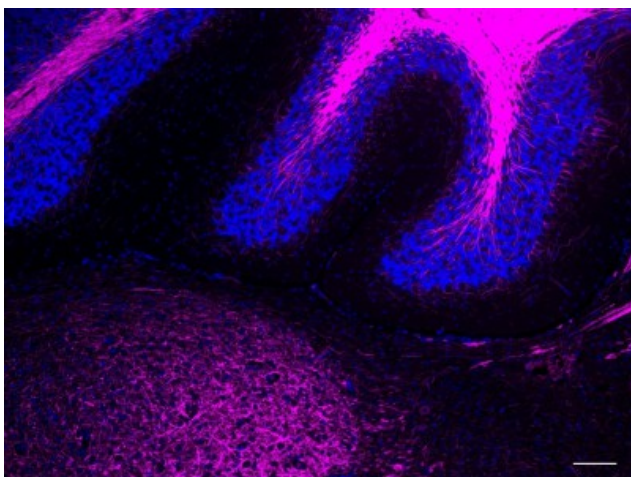
IHC staining of Alexa Fluor® 647 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated antibody (clone SMI 35) on formalin-fixed paraffin-embedded rat brain tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3 (Cat. No. 927601), the tissue was incubated with 10 µg/ml of the primary antibody overnight at 4°C. The image was captured with a 40X objective. Scale bar: 50 µm



IHC staining of Alexa Fluor® 647 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated antibody (clone SMI 35) on formalin-fixed paraffin-embedded human brain tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3 (Cat. No. 927601), the tissue was incubated with 10 µg/ml of the primary antibody overnight at 4°C. The image was captured with a 10X objective. Scale bar: 100 µm



IHC staining of Alexa Fluor® 647 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated antibody (clone SMI 35) on formalin-fixed paraffin-embedded mouse brain tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3 (Cat. No. 927601), the tissue was incubated with 10 µg/ml of the primary antibody overnight at 4°C. The image was captured with a 10X objective. Scale bar: 100 µm



IHC staining of Alexa Fluor® 647 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated antibody (clone SMI 35) on formalin-fixed paraffin-embedded rat brain tissue. Following antigen retrieval Retrieve-All Antigen Unmasking System 3 (Cat. No. 927601), the tissue was incubated with 10 µg/ml of the primary antibody overnight at 4°C. The image was captured with a 10X objective. Scale bar: 100 µm

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