Alexa Fluor® 647 anti-Neurofilament H (NF-H), Phosphorylated Antibody

Catalog# / Size	835505 / 25 µg 835506 / 100 µg
Clone	SMI 34
Regulatory Status	RUO
Other Names	Neurofilament heavy polypeptide, NF-H, 200 kD neurofilament protein, neurofilament triplet H protein
Isotype	Mouse IgG3, 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
Immunogen	Homogenized hypothalami from Fischer 344 rats
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	Each lot of this antibody is quality control tested by formalin-fixed paraffin-embedded immunohistochemical staining. For immunohistochemistry, a concentration range of 1.0 - 10.0 µg/mL is suggested. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633 nm / 635 nm. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Excitation Laser	Red Laser (633 nm)
Application Notes	SMI 34 reacts with a phosphorylated epitope in extensively phosphorylated neurofilament H and, to a lesser extent, with neurofilament M in most mammalian species, as well as in chicken.

Immunohistochemically, SMI 34 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 34. Staining is unaffected by trypsin. In pathological conditions, reaction with SMI 34 may be found also in neuronal cell bodies. In Alzheimer's disease, SMI 34 reacts with intraneuronal tangles but does not react with extraneuronal (ghost) tangles. It is the only anti-phosphoneurofilament that reacts regularly with Alzheimer tangles. It does not cross-react at concentrations of 1:20,000 with Alzheimer MAP-Tau in immunoblots, demonstrating, thereby, the presence of neurofilaments in Alzheimer tangles.

Additional Product Notes

For use in immunohistochemistry on formalin-fixed paraffin-embedded tissue (IHC-P), it is recommended to perform antigen retrieval using one of the following:

-Retrieve-All Antigen Unmasking System 3: Acidic, 100x (Cat. No. 927601)
-Citrate Buffer, 10X (Cat. No. 420902)
-Tris-EDTA pH 9.0 Antigen Retrieval Buffer (10X) (Cat. No. 422704).

Application References

(PubMed link indicates BioLegend citation)

1. Giasson BI, Mushynski WE. 1996. *J. Biol. Chem.* 271:30404.
2. Zhang H, *et al.* 1989. *Proc. Natl. Acad. Sci. USA* 86:8045.
3. Cork LC, *et al.* 1986. *J. Neuropathol. Exp. Neurol.* 45:56.
4. Sternberger NH, *et al.* 1985. *Proc. Natl. Acad. Sci. USA* 82:4274.
5. Shea TB, Beermann ML. 1993. *J. Neuroimmunol.* 1:117. (WB)
6. Mandelkow EM, *et al.* 1992. *Proc. Natl. Acad. Sci. USA* 12:5384. (WB)
7. Wataya T, *et al.* 2002. *J. Biol. Chem.* 7:4644. (IHC, WB)
8. Redondo J, *et al.* 2014. *Brain Pathol.* 25:692. (IF)
9. Giasson BI, Mushynski WE. 1997. *J. Neurosci.* 24:9466. (WB)
10. Duffy KR, Livingstone MS. 2005. *Cereb. Cortex* 8:1146. (IHC)

Antigen Details

Structure

Neurofilament H runs at 200-220 kD.

Distribution

Cytoplasmic/cytoskeleton

Interaction

SMI 34 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.

Cell Type

Mature Neurons

Biology Area

Cell Biology, Cell Motility/Cytoskeleton/Structure, Neuroscience, Neuroscience Cell Markers

Molecular Family

Intermediate Filaments, Phospho-Proteins

Antigen References

1. Giasson BI, Mushynski WE. 1996. *J Biol. Chem.* 271:30404.
2. Zhang H, *et al*. 1989. *Proc. Natl. Acad. Sci. USA* 86:8045.
3. Cork LC, *et al*. 1986. *J. Neuropathol. Exp. Neurol.* 45:56.
4. Sternberger NH, *et al*. 1985. *Proc. Natl. Acad. Sci. USA.* 82:4274.
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6. Mandelkow EM, *et al*. 1992. *Proc. Natl. Acad. Sci. USA 12:5384.* (WB)
7. Wataya T, *et al*. 2002. *J. Biol. Chem.* 7:4644. (IHC, WB)
8. Redondo J, *et al*. 2014. *Brain Pathol.* 25:692. (IF)
9. Giasson BI, Mushynski WE. 1997. *J. Neurosci.* 24:9466. (WB)
10. Duffy KR, Livingstone MS. 2005. *Cereb. Cortex* 8:1146. (IHC)

Gene ID

4744

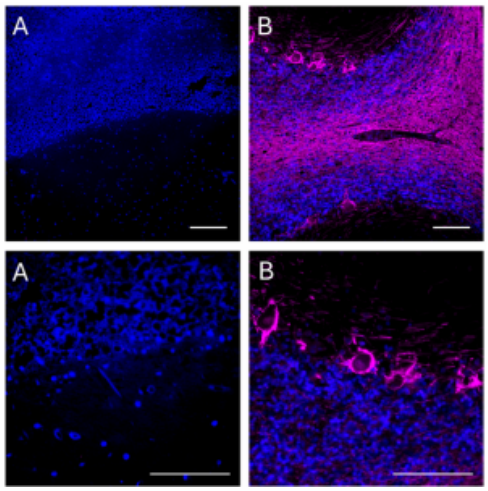
Related Protocols

- Immunohistochemistry Protocol for Paraffin-Embedded Sections

Other Formats

Alexa Fluor® 647 anti-Neurofilament H (NF-H), Phosphorylated, Purified anti-Neurofilament H (NF-H), Phosphorylated

Product Data



IHC staining of Alexa Fluor® 647 anti-Neurofilament H (NF-H), Phosphorylated (clone SMI 34) on formalin-fixed paraffin-embedded human cerebellum tissue. Following antigen retrieval using Retrieve-All Antigen Unmasking System 3: Acidic, 100x (Cat. No. 927601), the tissue was incubated without (panel A) and with (panel B) Alexa Fluor® 647 anti-Neurofilament H (NF-H), Phosphorylated (clone SMI 34). Nuclei were counterstained with DAPI (Cat. No. 422801). Images were captured with a 20x (upper panels) and 40X (lower panels) objective and merged. Scale bar: 50 µm

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