Purified anti-Neurofilament H (NF-H), Phosphorylated

Catalog # / Size: 801601 / 100 µl
Previously: Covance Catalog# SMI 31P
Clone: SMI 31
Isotype: Mouse IgG1, κ
Reactivity: Mammalian, Chicken, Xenopus
Preparation: The antibody was purified by affinity chromatography.
Formulation: Phosphate-buffered solution + Thimerosal.
Concentration: 1 mg/ml
Storage: The antibody solution should be stored undiluted between 2°C and 8°C. Please note the storage condition for this antibody has been changed from -20°C to between 2°C and 8°C. You can also check your vial or your CoA to find the most accurate storage condition for this antibody.

Applications:

Applications: IHC, WB, ICC, ELISA
Recommended Usage: Each lot of this antibody is quality control tested by immunohistochemical staining.

The optimal working dilution should be determined for each specific assay condition.
• WB: 1:1,000
• IHC: 1:500 - 1:2,000

Tissue Sections: Formalin-fixed, paraffin-embedded tissues & frozen sections
Pretreatment: For optimal staining, the sections should be pretreated with an antigen unmasking solution such as Retrieve-All 3 pH4.8 (Cat. No. 927601).
Incubation: 24 hours at 2-8°C using Biotin based detection systems such as USA Ultra Streptavidin Detection (Cat. No. 929501).

The extent of permissible dilution of SMI 31 beyond those recommended for general application depends upon nature and concentration of the antigen examined, species of the antigen, method of fixation and kind of section examined.

Application Notes: This antibody is effective in immunoblotting (WB), immunohistochemistry (IHC), immunocytochemistry, and ELISA.

Positive Control: Human cerebellum tissue

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:
1. Barry D, et al.; Expansion of Neurofilament Medium C Terminus Increases Axonal Diameter Independent of...
Neurofilaments (NF) are ~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. Neurofilaments belong to the same protein family as the intermediate filaments of other tissues such as keratins, which make the filaments expressed in epithelia. The family of proteins that includes the intermediate filaments is divided into 5 major classes, the keratins forming the classes I and II. Class III contains the proteins vimentin, desmin, peripherin and glial fibrillary acidic protein (GFAP). The major neurofilament subunits occupy the class IV family of intermediate filaments, along with two other filament proteins of neurons, alpha-internexin and nestin. The class IV intermediate filament genes share two unique introns not found in other intermediate filament gene sequences. Finally, class V corresponds to intermediate filaments of the nuclear cytoskeleton, the nuclear lamins. Neurofibrils are bundles of neurofilaments.

There are three major neurofilament 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.

These three proteins are referred to as the *neurofilament triplet*. Antibodies against neurofilaments are useful for identification of neurons and their processes in histological sections and in tissue culture. The true molecular masses of these proteins are considerably lower than estimated based on SDS-PAGE mobility, particularly in the case of NF-H and NF-M. This is due to the highly charged C-terminal regions of the molecules. All three triplet proteins contain long stretches of polypeptide sequence rich in glutamic acid residues, and NF-M and especially NF-H also contain multiple tandemly repeated serine phosphorylation sites. These sites contain the peptide sequence lysine-serine-proline, and phosphorylation is predominantly found on axonal and not dendritic mitochondria. Human NF-M has 13 of these KSP sites, while human NF-H contains 98 such sites. Human NF-L contains 16 KSP sites. The presence of this protein is widely used to define neurogenesis. This protein is lost as development proceeds.

The fourth class IV subunit, alpha-internexin (NF66) was discovered much later than NF-L, NF-M and NF-H, and is found co-polymerized with these proteins in mature neurons. The fifth protein belonging to class IV, Nestin, is found in developing neurons and glia, and the presence of this protein is widely used to define neurogenesis. This protein is lost as development proceeds.

The class III intermediate filament protein subunit peripherin is found in neurofilaments along with the class IV subunits in a few neurons, mostly in the peripheral nervous system. Finally another class III intermediate filament subunit, vimentin, is found in developing neurons and a few very unusual neurons in the adult in association with class IV proteins, such as the horizontal neurons of the retina.

In the adult mammal neurofilament subunit proteins coassemble, forming...
a heteropolymer that contain NF-L or alpha-internexin plus NF-M or NF-H. Peripherin and vimentin may incorporate into neurofilaments along with these proteins. The NF-H and NF-M proteins have lengthy C-terminal tail domains that appear to control the spacing between neighboring filaments, generating aligned arrays with a fairly uniform interfilament spacing as seen in axons.

During axonal growth, new neurofilament subunits are incorporated all along the axon in a dynamic process that involves the addition of subunits along the filament length, as well as the addition of subunits at filament ends. The level of neurofilament gene expression correlates with axonal diameter, which controls how fast electrical signals travel down the axon. Mutant mice with neurofilament abnormalities have phenotypes resembling amyotrophic lateral sclerosis. Neurofilament NF, immunostaining is common in diagnostic neuropathology. It is useful for differentiating neurons (positive for NF) from glia (negative for NF).

**Other Names:** Neurofilament heavy polypeptide, NF-H, 200 kD neurofilament protein, neurofilament triplet H protein, neurofilament triplet H protein

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