Neurofilament NF-H Rabbit Polyclonal Antibody

RPCA-NF-H

Ordering Information
Web *www.encorbio.com*
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Phone *352-372-7022*
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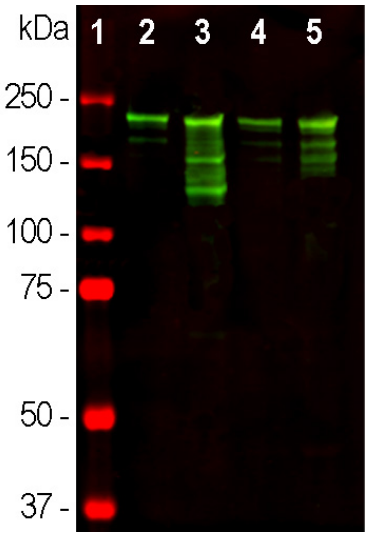
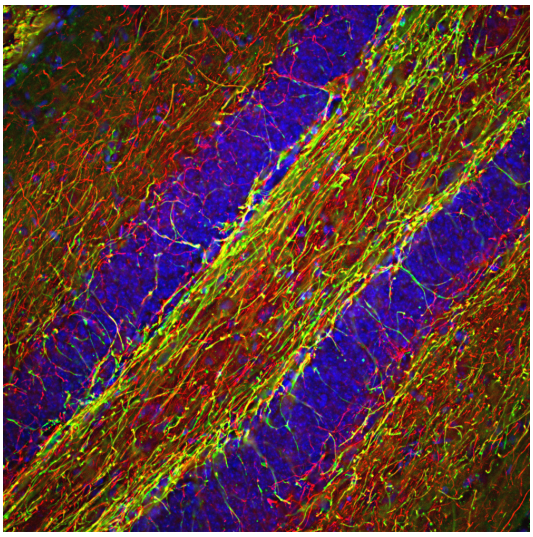
HGNC Name: *NEFH*
UniProt: *P12036*
RRID: *AB_2572360*
Immunogen: *Native NF-H purified from bovine spinal cord*
Format: *Antibody is supplied as an aliquot of serum plus 5mM NaN<>3*
Storage: *Store at 4°C. For long term storage, leave frozen at -20°C. Avoid freeze / thaw cycles.*
Recommended dilutions:
WB: 1:10,000-25,000. ICC/IF and IHC: 1:1,000-5,000.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Rabbit		200-220kDa	Hu, Rt, Ms, Co, Pi, Ho



Western blot analysis of different tissue lysates using rabbit pAb to NF-H, RPCA-NF-H, dilution 1:10,000 in green: [1] protein standard (red), [2] rat brain, [3] rat spinal cord [4] mouse brain, and [5] mouse spinal cord lysate. Strong band at about 220kDa corresponds to the phosphorylated axonal form of the NF-H subunit. Smaller proteolytic fragments of NF-H are also detected with RPCA-NF-H antibody.



Immunohistological analysis of a mouse hippocampus section stained with rabbit pAb to NF-H, RPCA-NF-H, dilution 1:2,000 in red, and costained with mouse mAb to myelin basic protein (MBP), MCA-7G7, dilution 1:5,000 in green. The blue is DAPI staining of nuclear DNA. Following transcardial perfusion with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45µM, and free-floating sections were stained with above antibodies. The NF-H antibody labels a network of axons of different neurons, while the MBP antibody stains myelin sheath around these axons.

Background:

Neurofilaments are the 10nm or intermediate filament proteins found specifically in neurons, and are composed predominantly of three major proteins called NF-L, NF-M and NF-H, though other proteins may also be present. NF-H is the neurofilament high or heavy molecular weight polypeptide and runs on SDS-PAGE gels at 200-220 kDa, with some variability across species boundaries. The protein is in reality much smaller in molecular size, about 110kDa (1,2). The unusual SDS-PAGE mobility is due partly to a very high content of charged amino acids, particularly glutamic acid rich regions, and the non-phosphorylated form runs on SDS-PAGE at about 160kDa. The predominant type of NF-H is the axonal form which is heavily serine phosphorylated on 40 or more tandemly repeated lysine-serine-proline (KSP) containing peptides (3-5). The phosphorylation of these peptides results in considerable further retardation on SDS-PAGE gels, so the heavily phosphorylated axonal form runs at 200-220kDa with some species variability. Antibodies to NF-H are useful for identifying axonal processes in tissue sections and in culture. NF-H antibodies can also be useful in visualizing neurofilament accumulations seen in many neurological disorders, such as Amyotrophic Lateral Sclerosis (also known as Lou Gehrig's disease), Alzheimer's disease and following traumatic injury. The phosphorylated axonal form of NF-H usually referred to as pNF-H, can be detected in blood and CSF following a variety of damage and disease states resulting in axonal compromise, and antibodies such as this can be used to used to quantify such ongoing axonal loss (e.g. 6-8).

The RPCA-NF-H antibody was raised against biochemically isolated NF-H purified from bovine spinal cord (9). This preparation is dominated by axonal forms of NF-H which are heavily phosphorylated on the multiply repeated NF-H KSP type sequences, and this antibody reacts very strongly with these phosphorylated repeats. Reactivity with non-phosphorylated KSP sequences is orders of magnitude weaker, similar to other characterized antibodies to NF-H (5). In most species there is some cross-reactivity with the phosphorylated KSP sequences found in the related neurofilament subunit NF-M which are similar but not identical to those of NF-H. The antibody recognizes phosphorylated NF-H strongly in all mammals tested to date and also in chicken. RPCA-NF-H recognizes neurofilaments in frozen sections, in tissue culture and in formalin fixed sections. We also supply three mouse monoclonal antibodies and a widely used chicken and goat polyclonal antibodies made to the same immunogen, MCA-NAP4, MCA-9B12, MCA-AH1, CPCA-NF-H and GPCA-NF-H.

References:

1. Perrot R, et al. Review of the Multiple Aspects of Neurofilament Functions, and their Possible Contribution to Neurodegeneration. Mol. Neurobiol. 38:27-65 (2008).
2. Lépinoux-Chambaud C. Eyer J. Review on intermediate filaments of the nervous system and their pathological alterations. Histochem. Cell Biol. 140:13-22 (2013).
3. Sternberger LA, Sternberger NH. Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. PNAS 80:6126-30 (1983).
4. Julien JP, Mushynski WE. Multiple phosphorylation sites in mammalian neurofilament polypeptides. J. Biol. Chem. 257:10467-70 (1982).
5. Lee VM, et al. Identification of the major multiphosphorylation site in mammalian neurofilaments. PNAS 85:1998-2002 (1988).
6. Shaw G, et al. Hyperphosphorylated neurofilament NF-H is a serum biomarker of axonal injury. Biochem. Biophys. Res. Commun. 336:1268-77 (2005).
7. Boylan et al, Immunoreactivity of the phosphorylated axonal neurofilament H subunit (pNF-H) in blood of ALS model rodents and ALS patients: evaluation of blood pNF-H as a potential ALS biomarker. J. Neurochem. 111:1182-91 (2009).
8. Shaw G. The Use and Potential of pNF-H as a General Blood Biomarker of Axonal Loss: An Immediate Application for CNS Injury. In: Kobeissy FH, editor. Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. CRC Press/Taylor & Francis; 2015. Chapter 21 .
9. Delacourte A, et al. Study of the 10-nm-filament fraction isolated during the standard microtubule preparation. Biochem. J. 191:543-6 (1980).

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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