

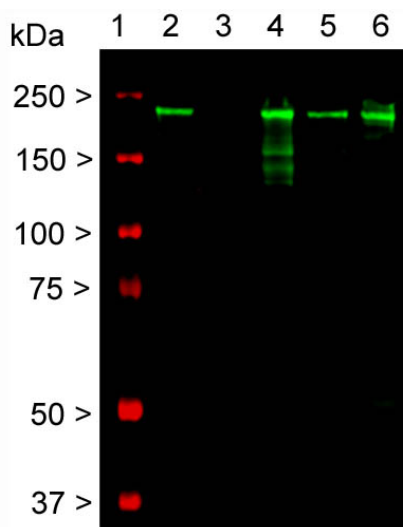
Ordering Information
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HGNC Name: NEFH
UniProt: P12036
RRID: AB_2572357
Immunogen: Native NF-H purified from bovine spinal cord
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaH₂PO₄
Storage: Store at 4°C. For long term storage, leave frozen at -20°C
Recommended dilutions:
WB: 1:10,000. ICC/IF: 1:1,000. IHC: 1:4,000.

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 JP, 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).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG1 heavy, κ light	200-220kDa	Hu, Rt, Ms, Co, Pi, Ho

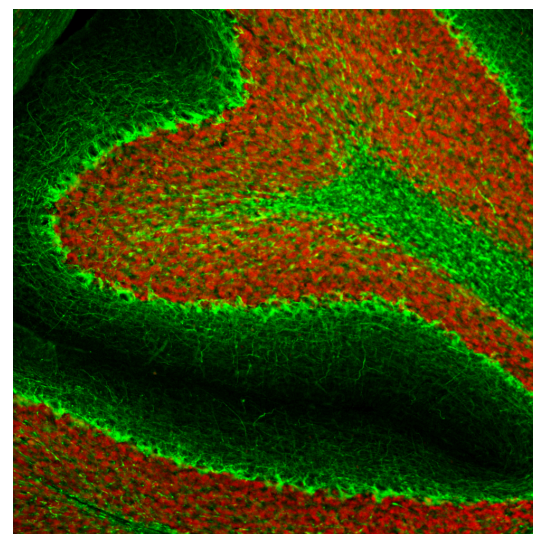


Western blot detection of the heavily phosphorylated axonal form of NF-H protein (pNF-H) in neural tissue lysates (20μg/lane) with affinity purified mouse monoclonal anti-pNF-H antibody (MCA-AH1) at dilution of 1:5,000. Lanes on the blot are: [1] Protein size marker, [2] Adult rat whole brain [3] Embryonic (E20) rat whole brain [4] Adult rat spinal cord [5] Adult mouse whole brain [6] Adult mouse spinal cord. Rodent pNF-H protein appears as a single band of about 200kDa in adult rat and mouse lysates, but is not present in early development (Lane 3). Additional bands appearing on the blot (Lane 4) are most likely partially degraded products of pNF-H protein.

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 quantify such ongoing axonal loss (e.g. 6-8).

MCA-AH1 is a mouse monoclonal antibody raised against native axonal phosphorylated NF-H purified from bovine spinal cord (9). MCA-AH1 recognizes phosphorylated NF-H KSP sequences but not non-phosphorylated KSP sequences, similar to other antibodies to NF-H (5,7). In some species there is some cross-reactivity with the phosphorylated KSP sequences found in the related neurofilament subunit NF-M. The antibody recognizes NF-H strongly on western blots of all mammals tested to date works well for IF, ICC and IHC (for IHC see data under "Additional Info" tab). It recognizes neurofilaments in frozen sections in tissue culture and in formalin fixed sections. We also market alternate mouse monoclonal antibodies to NF-H [MCA-NAP4](#) and [MCA-9B12](#) and also rabbit and chicken polyclonal antibodies [RPCA-NF-H](#) and [CPCA-NF-H](#), all of which have similar specificities to MCA-AH1. However MCA-AH1 was developed specifically by screening for a reagent which works well as in ELISA (7).



Immunohistological analysis of rat cerebellum section stained with mouse mAb to pNF-H, MCA-AH1, dilution 1:2,000 in green, and costained with rabbit pAb to FOX3/NeuN, [RPCA-FOX3](#), dilution 1:5,000 in red. 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 MCA-AH1 antibody stains axons in the granular layer and white matter and prominent basket cell axons surrounding the large Purkinje neurons. The FOX3/NeuN antibody specifically labels nuclei of granular and other neurons, but does not stain Purkinje cells.

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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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