EnCor Biotechnology Inc.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG2b heavy, κ light	200-220kDa	Hu, Rt, Ms

HGNC Name: NEFH
UniProt: P12036
RRID: AB_2572358
Immunogen: Native NF-H purified from bovine spinal cord, binding to phosphorylated KSP sequences
Format: Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN₃
Storage: Store at 4°C. For long term storage, leave frozen at -20°C. Avoid freeze / thaw cycles.
Recommended dilutions:
WB: 1:10,000. ICC/IF: 1:1,000. IHC: 1:10,000.

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 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).
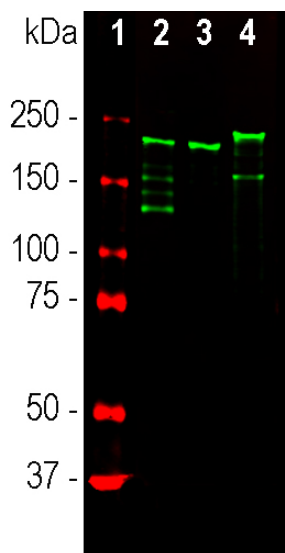
Western blot analysis of different tissue lysates using mouse mAb to NF-H, MCA-9B12, dilution 1:10,000 in green: [1] protein standard, [2] rat spinal cord [3] mouse spinal cord, and [4] cow spinal cord. Strong band at about 200-220kDa corresponds to the major phosphorylated form of the NF-H subunit. Smaller proteolytic fragments of NF-H are also detected in some preparations.
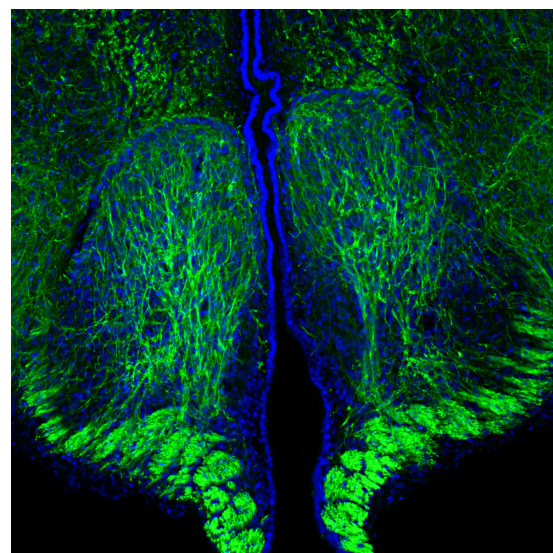
Immunohistological analysis of a rat brain coronal section of the third ventricle stained with mouse monoclonal antibody to phosphorylated NF-H, MCA-9B12, dilution 1:5,000 in green. The blue is Hoechst staining of nuclear DNA. Following transcardial perfusion with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45μM, and free-floating sections were stained with above antibody. The MCA-9B12 antibody is a robust marker of the axons of neuronal cells.

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 160-220 kDa, with some variability across species boundaries though in reality is much smaller, about 110kDa (1,2). The unusual SDS-PAGE mobility is due to a very high content of negatively charged amino acids 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 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 neurological disorders, such as amyotrophic lateral sclerosis, 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).

MCA-9B12 is a mouse monoclonal antibody raised against native axonal phosphorylated NF-H purified from bovine spinal cord (9). MCA-9B12 recognizes the phosphorylated NF-H KSP sequences similar to other antibodies to NF-H (5,7). There is some cross-reactivity with the phosphorylated KSP sequences found in the related neurofilament subunit NF-M. The antibody recognizes NF-H strongly in all mammals tested to date and also in chicken. The antibody works well for western blotting and for IF, ICC and IHC (for IHC see data under "Additional Info" tab). We also market alternate mouse monoclonal antibodies to NF-H MCA-NAP4 and MCA-AH1 and also rabbit and chicken polyclonals RPCA-NF-H and CPCA-NF-H, all of which have similar specificities to MCA-9B12.

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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