

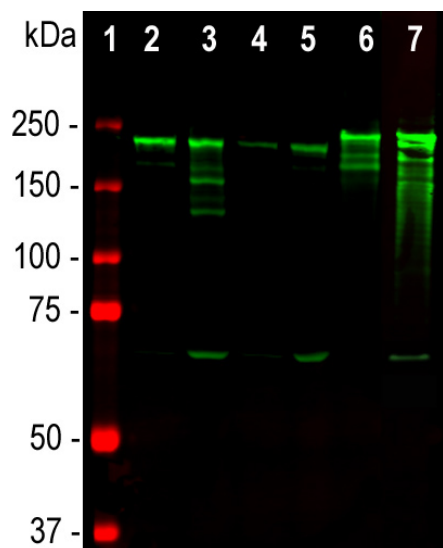
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HGNC Name: NEFH
UniProt: F1MSQ6
RRID: AB_2572359
Immunogen: Native NF-H purified from bovine spinal cord
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaCl,
Storage: Store at 4°C for short term, for longer term at -20°C
Recommended dilutions:
 WB: 1:10,000. IF/ICC: 1:1,000. IHC: 1:10,000

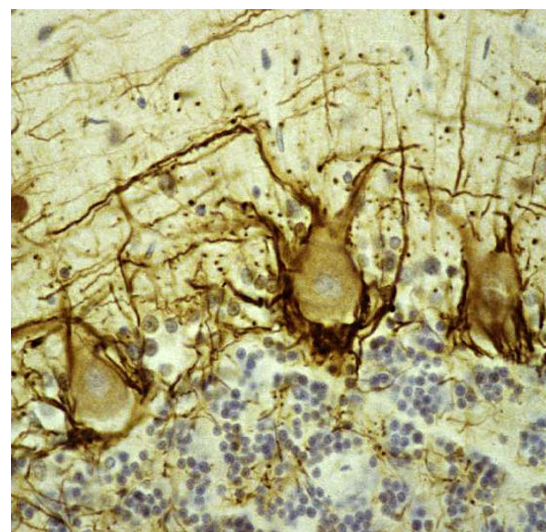
References:

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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, Ck



Western blot analysis of tissue lysates using mouse mAb to NF-H, MCA-NAP4, dilution 1:10,000 in green: [1] protein standard (red), [2] rat brain, [3] rat spinal cord, [4] mouse brain, [5] mouse spinal cord, [6] pig spinal cord, [7] cow spinal cord. Strong band at about 200-220 kDa corresponds to the major phosphorylated form of the NF-H subunit. A minor band at about 160 kDa is the non-phosphorylated NF-H. Smaller proteolytic fragments of NF-H are also detected in spinal cord preparations with MCA-NAP4 antibody.



Immunohistological analysis of human cerebellar cortex section stained with mouse mAb to pNF-H, MCA-NAP4, in brown. Paraffin-embedded, formalin-fixed tissue sections were stained with this antibody using the avidin biotin conjugate method. The sections were counterstained with Hematoxylin in blue. MCA-NAP4 stains prominent basket cell axons surrounding the large Purkinje neurons. Cerebellar granule cell layer is at the bottom of the image, the molecular layer at the top.

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-NAP4 is a mouse monoclonal antibody raised against native axonal phosphorylated NF-H purified from bovine spinal cord (9). MCA-NAP4 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 in all mammals tested to date and also in chicken. 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-9B12](#) and [MCA-AH1](#) and also rabbit and chicken polyclonal antibodies [RPCA-NF-H](#) and [CPCA-NF-H](#), all of which have similar specificities to MCA-NAP4.

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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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