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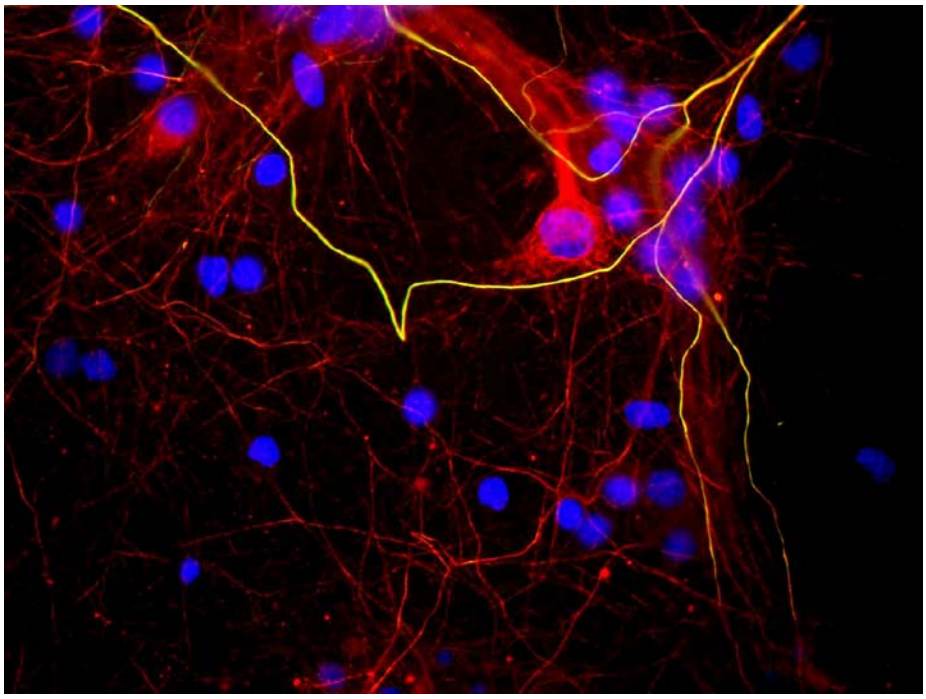
Catalogue# MCA-AH1: Neurofilament NF-H Monoclonal Antibody AH1

The Immunogen: 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. 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.

Antibodies to NF-H are useful for identifying neuronal cells and their processes in tissue sections and in tissue culture. NF-H antibodies can also be useful in the diagnostics of neurofilament accumulations seen in many neurological diseases, such as Amyotrophic Lateral Sclerosis (also known as Lou Gehrig's disease) and Alzheimer's disease.

MCA-AH1 is one of numerous antibodies which reacts preferentially with the axonal phosphorylated forms of NF-H. Interestingly these phosphorylated forms of NF-H are normally restricted to axons, while less phosphorylated forms are found in dendrites. However in numerous damage and disease states, phosphorylated NF-H can be detected with MCA-AH1 in dendritic and perikaryal neurofilaments. The HGNC name for this protein is NEFH. The characterization of this antibody has been published (1).



Left: Strip blots of crude rat spinal cord extract stained with three different antibodies to phosphorylated NF-H, MCA-NAP4 (lane 1), MCA-AH1 (Lane 2) and MCA-9B12 (lane 3). All three antibodies bind to a prominent band with an apparent SDS-PAGE molecular weight of 200 kDa. **Right:** Mixed neuron/glial cultures stained with MCA-AH1 (green) and also stained with rabbit polyclonal antibody to neurofilament NF-L RPCA-NF-L (red). The NF-L antibody stains neurofilaments in both axons and dendrites, and so can reveal neuronal cell bodies, while MCA-AH1 binds to only heavily phosphorylated forms of NF-H which are localized to mature axonal neurofilaments. In this image a few axons course from left to right and top to bottom- since they contain both NF-L and phosphorylated NF-H they appear golden in color. Blue shows the distribution of DNA.

Antibody characteristics: MCA-AH1 is a mouse monoclonal antibody raised against a preparation of native NF-H purified from bovine spinal cord. MCA-AH1 is an IgG1 class antibody with a k light chain. It recognizes phosphorylated NF-H KSP (lysine-serine-proline) type sequences. In some species there is some cross-reactivity with the related 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.

Suggestions for use: The ascites solution has a high titer and can be used at dilutions of at least 1:500 in immunofluorescence experiments. As with many ascites preparations there is a small immune response to bovine serum albumin, since the cells used to generate the ascites were grown in this. We therefore recommend the use of non fat milk as a blocking agent for western blots, ELISA etc. In western blotting using chemiluminescence MCA-AH1 can be used at dilutions of 1:5,000 or lower. Antibody preparation contains 10mM sodium azide preservative (Link to http://www.encorbio.com/MSDS/azide.htm for Material Safety Data Sheet). Store at 4°C short term or -20°C long term. Avoid repeated freezing and thawing. We also do have an affinity purified preparation at 1 mg/mL in PBS.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

References:

1. Boylan, K. 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. 111:1182-1191 (2009).

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