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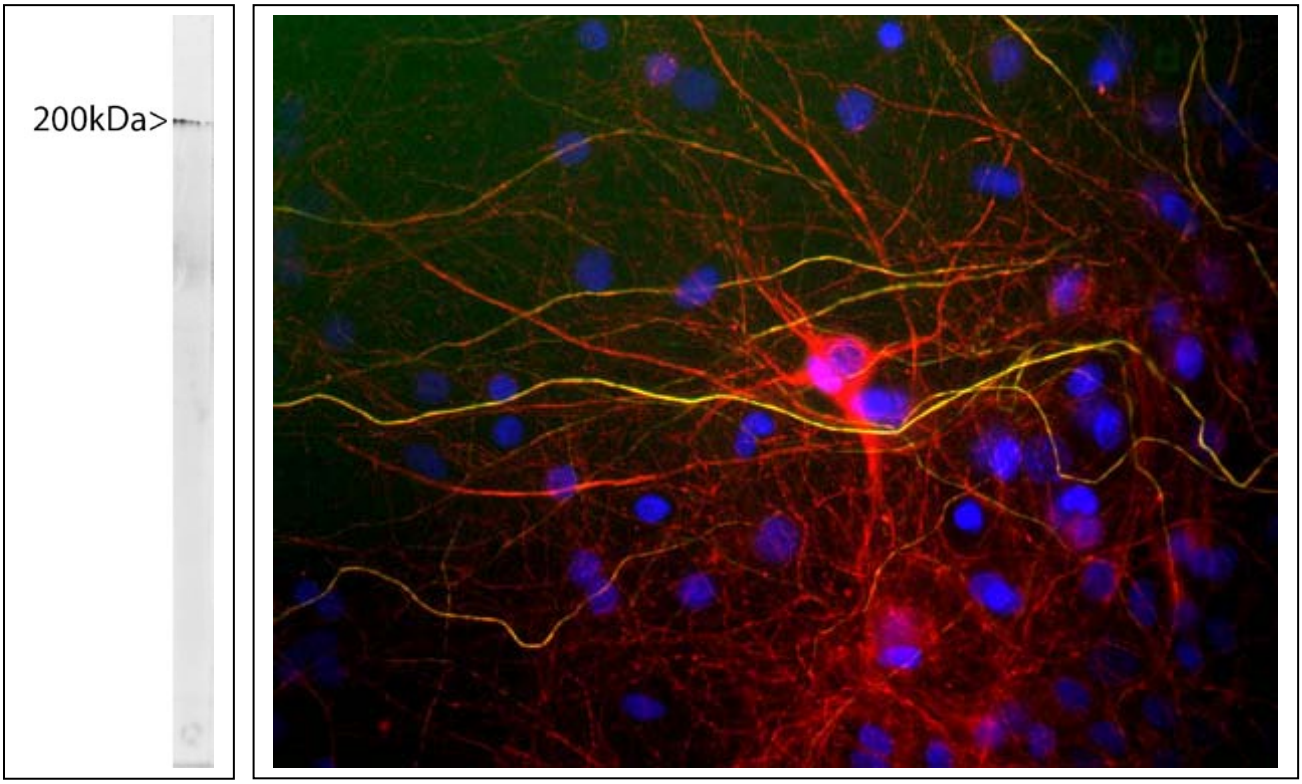
Catalogue# RPCA-NF-H: Rabbit Polyclonal Antibody to Neurofilament NF-H

The Immunogen: Neurofilaments can be defined as the intermediate or 10nm diameter filaments found in neuronal cells. They are composed a mixture of subunits which often includes the neurofilament triplet proteins, NF-L, NF-M and NF-H. Neurofilaments may also include peripherin, α-internexin, nestin and in some cases vimentin. Antibodies to the various neurofilament subunits are very useful cell type markers since the proteins are quite abundant, biochemically stable. To raise this antibody bovine intermediate filaments were prepared from spinal cords by the method of Delacourte et al. and the cytoskeletal material was dissolved in 6M urea. Individual neurofilament subunits were purified by ion exchange chromatography on DEAE cellulose followed by preparative gel electrophoresis. The form of NF-H isolated is the heavily phosphorylated axonal variant, and the antibody generated recognizes predominantly axonal neurofilaments.
The HGNC name for this protein is NEFH.



Left: Western blot of crude rat spinal cord extract blotted with RPCA-NF-H, revealing a single strong band at 200 kDa, the size expected for axonal phosphorylated forms of NF-H. **Right**: Mixed neuron/glia cultures stained with RPCA-NF-H (green) and CPCA-NF-M, chicken antibody to neurofilament subunit NF-M. Axons contain phosphorylated NF-H and NF-M so appear yellowish, while dendrites and perikarya only contain NF-M and so appear red. DNA is shown in blue.

Antibody Characteristics: This antibody was generated in rabbit against purified bovine NF-H. Bovine intermediate filaments were prepared from spinal cords by the glycerol polymerization method of Delacourte et al. (1), and the cytoskeletal material was dissolved in 6M urea. Individual neurofilament subunits were purified by ion exchange chromatography on DEAE cellulose followed by preparative gel electrophoresis on a Biorad Prepcell. Antibody should stain a protein band of apparent SDS-PAGE molecular weight 200-220 kDa, with generally smaller species (i.e. rat and mouse) showing a ~200 kDa protein bands while larger species (i.e. cow, pig and human) having a larger ~220 kDa band (2). Antibody recognizes axonal neurofilaments particularly

well. Antibody has also been successfully used, following affinity purification, for NF-H detection in an ELISA capture assay (2). Store at 4°C or -20°C. Avoid repeat freezing and thawing. The characterization of this antibody has not been formally published, though is similar to the antibody described in reference 2.

Suggestions for use: We suggest a dilution of 1:1,000 for immunofluorescence microscopy and 1:10,000 for western blots using chemiluminescence. Recognizes primarily the 200-220 kDa hyperphosphorylated axonal form of NF-H.

References:

1. Delacourte A, Filliatreau G, Boutteau F, Biserte G, Schrevel J. Study of the 10-nm- filament fraction isolated during the standard microtubule preparation. Biochem J. 191:543-6 (1980).

2. Shaw G, Yang C, Ellis R, Anderson K, Parker Mickle J, Scheff S, Pike B, Anderson DK and Howland DR. Hyperphosphorylated neurofilament NF-H is a serum biomarker of axonal injury. Biochem Biophys Res Commun. 336:1268-1277 (2005).

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