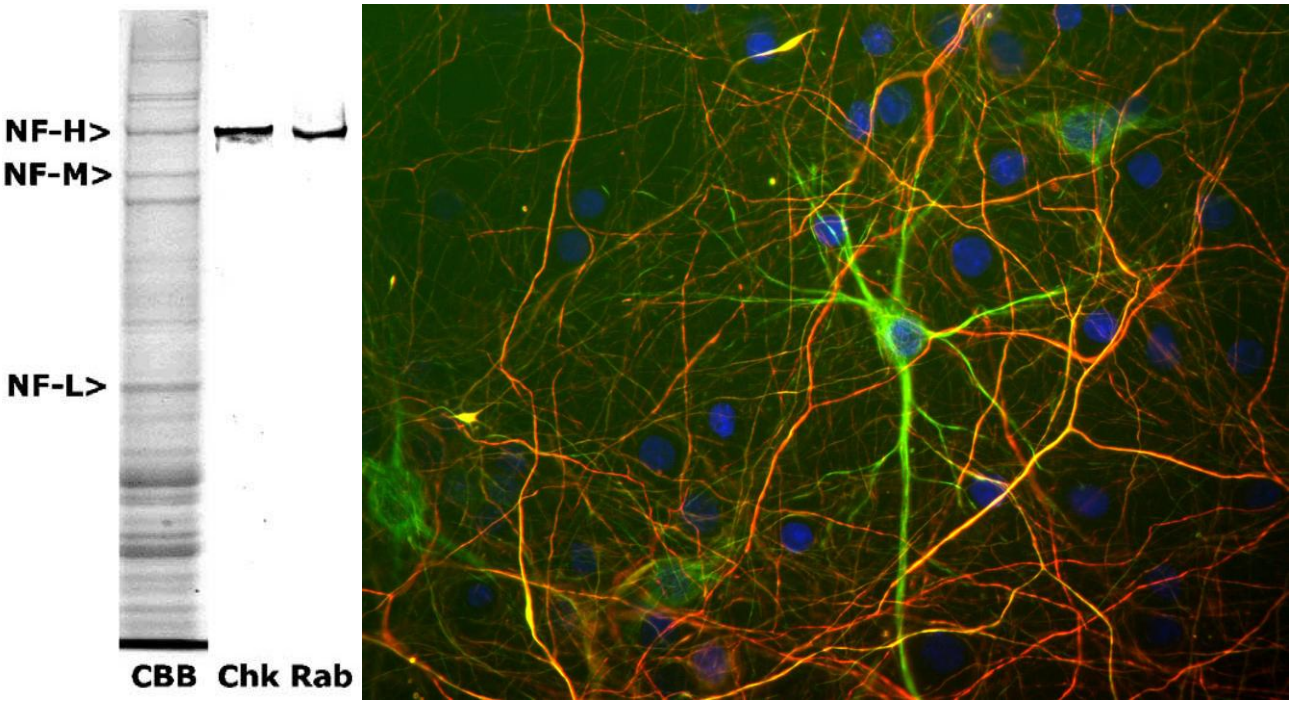
4949 SW 41st Blvd.
Suites 40 & 50
Gainesville, FL 32608
Tel: (352) 372 7022
Fax: (352) 372 7066
admin@encorbio.com

Catalogue# CPCA-NF-H: Chicken Polyclonal Antibody to Neurofilament NF-H

The Immunogen: Neurofilaments can be defined as the intermediate or 10nm filaments found in specifically in neuronal cells. In the electron microscope neurofilaments appears as 10nm diameter fibers of indeterminate length which generally have fine wispy protrusions from their sides. They are found particularly abundantly in axons of large projection neurons. They probably function to provide structural support for neurons and their synapses and to support the large axon diameters required for rapid conduction of impulses down axons. They are composed a mixture of subunits which usually include the three neurofilament triplet proteins, known as NF-L, NF-M and NF-H. Neurofilaments may also include smaller amounts of 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 among the most abundant of the nervous system, are expressed only in neurons, and are biochemically very stable. To raise this particular antibody bovine intermediate filaments were prepared from spinal cords by the glycerol polymerization 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 on a Biorad Prepcell. The HGNC name for this protein is NEFH.



Figures: **Left:** CBB shows lane of Coomassie Brilliant Blue stained crude extract of rat spinal cord, with the prominent major neurofilament subunit indicated; In rodents, NF-H runs at ~200kDa, NF-M at ~145kDa and NF-L at ~68kDa; in larger species such as pig or human, NF-H runs somewhat slower at ~210kDa or 220kDa. The Chk lane shows a blot of the same material probed with chicken anti NF-H antibody CPCA-NF-H. A prominent band at an apparent SDS-PAGE molecular weight of 200kDa corresponds to phosphorylated form of NF-H. The lane marked Rab shows a similar blot probed with our rabbit antibody to NF-H, RPCA-NF-H. **Right:** shows rat mixed neuron/glial cultures stained with mouse monoclonal antibody to neurofilament subunit NF-L MCA-7D1 (green) and CPCA-NF-H, EnCor's chicken antibody to neurofilament NF-H. This antibody binds primarily to the phosphorylated axonal forms of NF-H, in contrast to the NF-L antibody which stains both axonal and dendritic/perikaryal neurofilaments. The NF-L antibody therefore reveals a prominent cell body in green, while the surrounding axonal profiles are orange, since they are bound by both NF-L and the chicken NF-H antibody. Blue is a DNA stain.

Antibody Characteristics: On western blots CPCA-NF-H recognizes NF-H from all mammalian species tested to date very strongly and shows some weaker reactivity with NF-M. The most immunogenic regions of NF-H and NF-M are the phosphorylated KSP type repeats, and this antibody shows very strong reactivity with these. This antibody was generated in chicken by standard procedures and immunoglobulin was extracted from egg yolk. The resulting polyclonal antibody belongs to the IgY subclass. This is the chicken homologue of mammalian IgG and can be used in the same general way, with the caveat that this type of antibody does not bind either Protein A or Protein G. Suitable second antibody reagents can be obtained from many vendors including Molecular Probes and Sigma-Aldrich. 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 or -20°C. Avoid repeat freezing and thawing. The current batch of this antibody, as of the date below, is 2796.

Suggestions for use: The IgY solution has an extremely high titer and can be used at dilutions of at least 1:100,000 in immunofluorescence experiments. The total protein concentration of the current batch is about 25mg/mL. In western blotting using chemiluminescence it can be used at dilutions of 1:1,000,000 or lower. We supply aliquots of 50 μL, which should be enough for thousands of experiments.

Examples: For some on line images generated with this antibody go to http://www.encorbio.com/Album/Chickenabpics.htm.

References:

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