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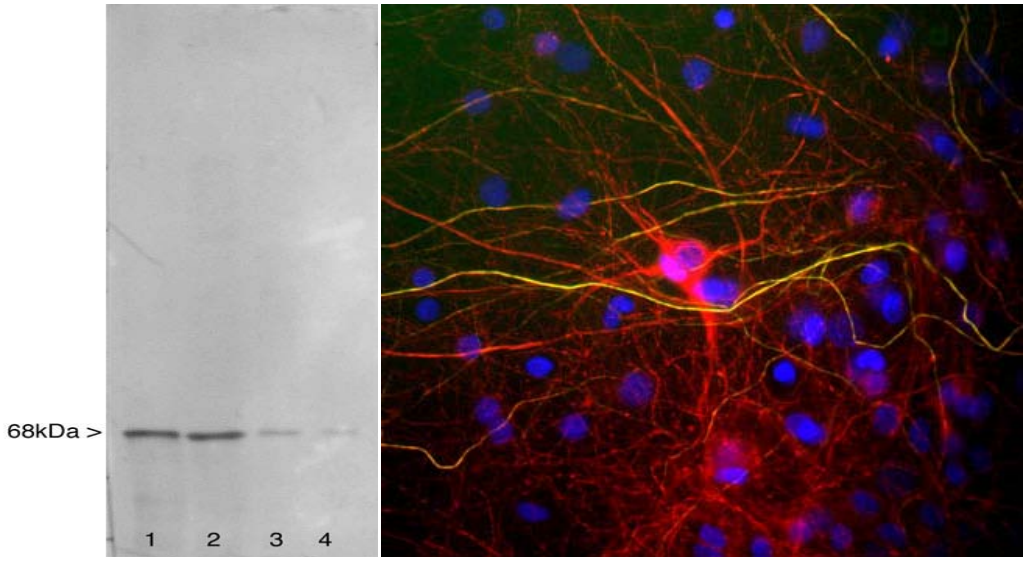
Catalogue CPCA-NF-L: Chicken Polyclonal Antibody to Neurofilament NF-L

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 fibres 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 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. To ensure greater specificity for NF-L, animals were boosted with recombinant mouse NF-L purified from bacteria. The HGNC name for this protein is NEFH.



Left: Western blots of urea extracts of rat spinal cord (lane 1), brain stem (lane 2), cerebellum (lane 3) and cerebral cortex (lane 4). Neurofilaments are concentrated in large projection axons and therefore NF-L is a much more major component of spinal cord than cortical regions (see reference). **Right:** View of mixed neuron/glial cultures stained with CPCA-NF-L (red) and EnCor's rabbit antibody to phosphorylated NF-H (RPCA-NF-H, green). The NF-L protein is assembled into neurofilaments which are found throughout the axons, dendrites and perikarya of these cells. In contrast the phosphorylated NF-H has a much more restricted expression pattern, being found only in developed axonal neurofilaments. Since both proteins are found in neurofilaments, the red and green patterns overlap, so that neurofilaments containing NF-L and phosphorylated NF-H appear yellowish. In contrast neurofilaments containing only NF-L appear red.

Antibody Characteristics: 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. The IgY preparation was made by chloroform delipidation of egg yolk followed by polyethylene glycol precipitation. The IgY total concentration is

21.5 mg/mL in phosphate buffered saline plus 10mM sodium azide preservative. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: The IgY preparation has a concentration of ~20mg/mL. The IgY solution can be used at dilutions of at least 1:5,000 in immunofluorescence experiments. In western blotting using chemiluminescence it can be used at dilutions of 1:10,000 or lower. The solution has a total protein concentration of 21.5 mg/mL in phosphate buffered saline plus 10mM sodium azide.

References:

1. 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.