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## Catalogue# RPCA-NF-H-pInd: Rabbit Polyclonal Antibody to Neurofilament NF-H (phosphate independent)

The Immunogen: Neurofilaments can be defined as the intermediate or 10nm diameter filaments found in neurons. 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,  $\alpha$ -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. The form of NF-H expressed in neurons varies in terms of the level of phosphorylation. Mammalian NF-H contains tandem repeats based on the sequence lysine serine proline (KSP), which may be present up to 60 times, with some variability between species. These KSP sequences are the sites of phosphorylation, and in axons most if not all of the serine residues are phosphorylated. In contrast, neurofilaments in dendrites and perikarya are not phosphorylated on these KSP sequences. Axonal neurofilaments are therefore quite different immunologically from those in perikarya and dendrites, and many antibodies stain only axonal or only dendritic/perikaryal neurofilaments. This antibody was raised against the KSP region of rat NF-H which was expressed in and purified from *E. coli*. It is similar to, but higher quality than, an antibody made and described many years ago (1). Since bacteria do not phosphorylate mammalian proteins, this protein resembles the NF-H form characteristic of perikaryal and dendritic neurofilaments. Accordingly this antibody stains dendritic and perikaryal neurofilaments particularly well. The HGNC name for this protein is NEFH.



**Left:** Blot of rat spinal cord extract blotted with RPCA-NF-H-Pind. A major band running at 200 kDa is NF-H, the major neurofilament subunit protein. A minor band at about 160 kDa is the non-phosphorylated dendritic and perikaryal form of this protein. **Right:** Mixed neuron/glial rat brain cultures stained with RPCA-NF-H-Pind (green) and EnCor's chicken polyclonal antibody to Glial Fibrillary Acidic Protein (GFAP) <u>CPCA-GFAP</u> (red). The neurofilament NF-H antibody binds to phosphorylated axonal forms of NF-H and non-phosphorylated dendritic and perikaryal forms.

**Antibody Characteristics:** Antibody was raised in rabbit against a purified NF-H construct from a rat cDNA which encoded most of the tandem KSP phosphorylation site repeats. Antibody is available as an aliquot of crude serum. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

**Suggestions for use:** We suggest a dilution of 1:1,000 for immunofluorescence microscopy and 1:10,000 for western blots. The antibody has also been used successfully in the detection role in ELISA.

## **References:**

1. Harris, J., Ayyub, C. and Shaw G. A molecular dissection of the carboxyterminal tails of the major neurofilament subunits NF-M and NF-H. <u>J Neurosci Res 30:47-62 1991.</u>

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

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