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HGNC name: NEFL RRID: AB 2149931

Immunogen: Recombinant human NF-L protein

Format: Antibody is supplied as an aliquot of concentrated IgY prep. Storage: Shipped on ice, storage at 4°C recommended. For longer term storage make 50% wrt glycerol and put at -20°C

Recommended dilutions: Western blot: 1:5,000.

IF/ICC and IHC: 1:2,000.

References:

1. Hoffman.et al. Neurofilament gene expression:a major determinant of axonal caliber. PNAS USA.84:3472-3476 (1987).

2. Liu Q. et al. Neurofilamentopathy in Neurodegenerative Diseases. Open Neurol. J. 5:58–62 (2011).

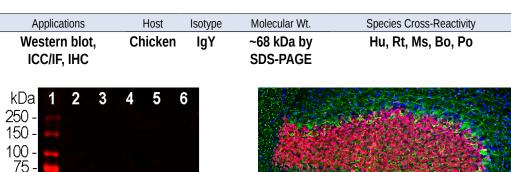
3. Mersiyanova IV. et al. A new variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene. Am. J. Hum. Genet. 67:37-46, 2000.

4. Braissant O. Neurofilament Proteins in Brain Diseases. In: New Research on Neurofilament Proteins. Chapter II:25-51 (2007)

5. Rana O.R.et al. Neurofilament light chain as an early and sensitive predictor of long-term neurological outcome in patients after cardiac arrest. Int. J. of Cardiol. 168:1322-1327 (2013)

Chicken pAb to NF-L



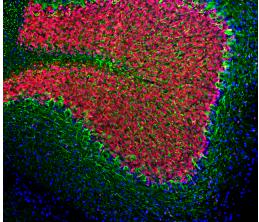


Western blot analysis of tissue lysates probed with chicken pAb to NF-L, CPCA-NF-L, dilution 1:20,000 in green: [1] protein standard (red), [2] rat brain, [3] rat spinal cord, [4] mouse brain, [5] mouse spinal cord and [6] cow spinal cord. Strong bands at ~68kDa corresponds to NF-L proteins which are known to have slightly different apparent SDS-PAGE molecular weights across species boundaries.

50

37.

25



Immunofluorescent analysis of rat cerebellum section stained with chicken pAb to NF-L, CPCA-NF-L, dilution 1:2,000 in green, and costained with mouse mAb to FOX3/NeuN, MCA-1B7, dilution 1:5,000 in red. Following transcardial perfusion of rat with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45µM, and free-floating sections were stained with above antibodies. CPCA-NF-L antibody labels perikarya and processes of neuronal cells, particularly strongly the axons of basket cells, while the FOX3/NeuN antibody stains the nuclei and proximal cytoplasm of neurons.

Background: Neurofilaments can be defined as the intermediate or 10nm filaments found in specifically in neuronal cells. In the electron microscope neurofilaments appear as 10nm diameter fibres of indeterminate length which generally have fine wispy protrusions from their sides. They are found to be particularly abundant in axons of large projection neurons (1). 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 as 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 animals were injected with recombinant mouse NF-L purified from bacteria. Much interest has been focused on this and other neurofilament proteins since mutations in the relevant genes are associated with certain neurological disorders and because detection of certain of these proteins in blood and CSF can be employed as a surrogate marker on CNS injury and disease states (e.g. 2-5).

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

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Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Bo—Cow Po—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli. followed by polyethylene glycol precipitation. The IgY total concentration is in the range of 20-30mg/mL in phosphate buffered saline plus 10mM sodium azide preservative.