

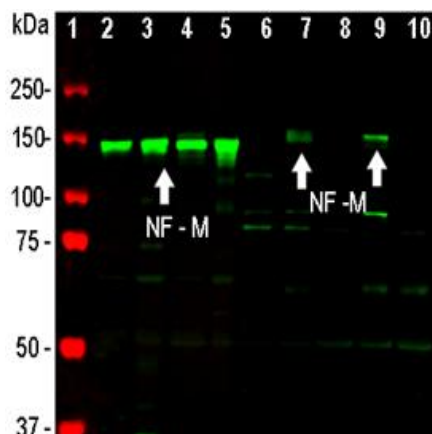
**Ordering Information**  
**Web** www.encorbio.com  
**Email** admin@encorbio.com  
**Phone** 352-372-7022

**HGNC name:** NEFM  
**RRID:** AB\_2572367  
**Host Species:** Chicken  
**Immunogen:** C-terminal extension of rat NF-M, the so-called KE segment, was expressed in bacteria and purified from inclusion bodies  
**Format:** concentrated IgY prep in PBS with 0.02% NaN<sub>3</sub> (total concentration is 26mg/mL)  
**Storage:** Store at 4°C for short term and at -20°C for long term. Avoid repeated freeze / thaw cycles.  
**Recommended dilutions:**  
 WB: 1:5,000 for neural tissue extracts, and 1:1000 for cells.  
 IF/IHC: 1:1,000  
**Application notes:** ICC/IF neuronal marker

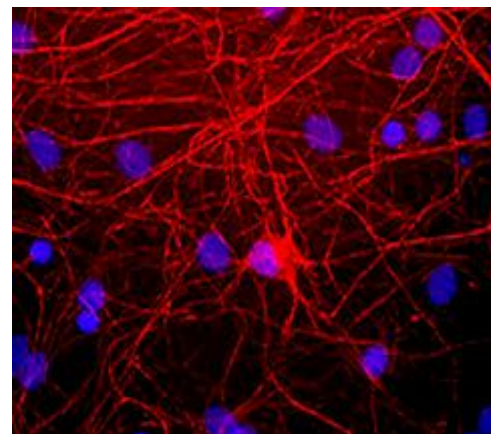
### References:

- Harris, J., Ayyub, C. and Shaw G. A molecular dissection of the carboxyterminal tails of the major neurofilament subunits NF-M and NF-H. *J Neurosci Res.* 1991 Sep;30(1):47-62.
- Shaw G, Yang C, Zhang L, Cook P, Pike B, Hill WD. Characterization of the bovine neurofilament NF-M protein and cDNA sequence, and identification of in vitro and in vivo calpain cleavage sites. *Biochem Biophys Res Commun.* 2004 Dec 10;325(2):619-25.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, ICC/IF, IHC	C	IgY	145 -160 kDa	H, M, R, C, B, P



**Western blot analysis of neural tissue and cell lysates using Chicken pAb against NF-M (CPCA-NF-M, green).** [1] protein standard, [2] Rat whole brain, [3] Rat spinal cord, [4] Mouse whole brain, [5] Mouse spinal cord, [6] NIH/3T3, [7] Hek293, [8] HeLa, [9] SH-SY5Y, [10] C6 cells. CPCA-NF-M antibody detects 145 kDa protein corresponding to rodent NF-M, and slightly higher molecular weight human NF-M in Hek293 and SH-SY5Y cells.



**Immunofluorescent analysis of rat neuron/glia cell cultures stained with Chicken pAb against NF-M (CPCA-NF-M, red).** The NF-M protein is assembled into neurofilaments which are found throughout the axons, dendrites, and perikarya of neurons. The blue is DAPI staining of nuclear DNA.

**Background:** **Neurofilaments** can be defined as the intermediate or 10nm filaments found in neuronal cells. In the electron microscope, neurofilaments appear as 10nm-diameter fibers of indeterminate length which generally have fine wispy protrusions from their sides. They are found abundantly in axons of large projection neurons. The possible function of neurofilaments is 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 three neurofilament triplet proteins known as NF-L, NF-M, and NF-H. Neurofilaments may also include smaller amounts of peripherin,  $\alpha$ -internexin, nestin and in some cases, vimentin. NF-M is the neurofilament middle or medium molecular weight polypeptide and appears on SDS-PAGE gels at 145-160 kDa.

Antibodies to NF-M are very useful cell type markers since the protein is among the most abundant of the nervous system, is expressed only in neurons, and is biochemically very stable. NF-M antibodies can also be useful to visualize the neurofilament aggregates seen in many neurological diseases, such as Amyotrophic Lateral Sclerosis and Alzheimer's disease.

To raise the anti-NF-M antibody, the C-terminal extension of rat NF-M, the so-called KE segment, was expressed in bacteria and purified from inclusion bodies by dissolving in 6M urea followed by preparative gel electrophoresis (1). CPCA-NF-M 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.

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### Abbreviation Key:

mAb—monoclonal antibody pAb—polyclonal antibody WB—Western IF—Immunofluorescence IHC—Immunohistochemistry ICC—Immunocytochemistry E—ELISA H—human M—mouse R—rat C—chicken B—bovine P—porcine D—dog