# EnCor Biotechnology Inc.

# Neurofilament NF-M Mouse Monoclonal Antibody

# MCA-3H11

**HGNC Name:** NEFM
**UniProt:** P07197
**RRID:** AB_2572365
**Immunogen:** Recombinant fusion protein containing the extreme C-terminus of rat NF-M, amino acids 677-845, expressed in and purified from E. coli
**Format:** Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN$_3$
**Storage:** Store at 4°C for short term, for longer term at -20°C
**Recommended dilutions:**
WB: 1:5,000. IF/ICC 1:2,000. IHC: 1:10,000.

**References:**

1. Hoffman et al. Neurofilament gene expression:a major determinant of axonal caliber. PNAS 84:3472-6 (1987).
2. Perrot R, et al. Review of the Multiple Aspects of Neurofilament Functions, and their Possible Contribution to Neurodegeneration. Mol. Neurobiol. 38:27-65 (2008).
3. Lépinoux-Chambaud C. Eyer J. Review on intermediate filaments of the nervous system and their pathological alterations. Histochem. Cell Biol. 140:13-22 (2013).
4. Liu Q. et al. Neurofilamentopathy in Neurodegenerative Diseases. Open Neurol. J. 5:58–62 (2011).
5. Bacioglu M, et al. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 91:56-66 (2016).
6. Shaw G. The use and potential of pNF-H as a general blood biomarker of axonal loss: an immediate application for CNS injury. in Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. CRC Press/Taylor & Francis Chapter 21 (2015).
7. 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. 30:47-62 (1991).
8. Shaw G. Identification of previously unrecognized sequence motifs at the extreme carboxyterminus of the neurofilament subunit NF-M. BBRC 14;162:294-9 (1989).
9. Shaw G, et al. Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. FASEB J. 16:869-71 (2002).

| Applications | Host | Isotype | Molecular Wt. | Species Cross-Reactivity |
|---|---|---|---|---|
| WB, IF/ICC, IHC | Mouse | IgG1 | 145-160kDa | Hu, Rt, Ms, Co, Pi, Ho, Ck |
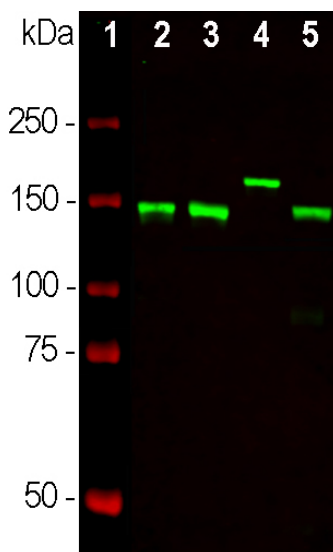


Western blot analysis of neuronal tissue lysates using mouse mAb to NF-M, MCA-3H11, dilution 1:10,000 in green: [1] protein standard (red), [2] rat spinal cord, [3] mouse spinal cord, [4] cow spinal cord and [5] rat sciatic nerve. Strong bands at 145kDa correspond to rodent NF-M while that at about 160kDa corresponds to the significantly larger bovine NF-M protein.
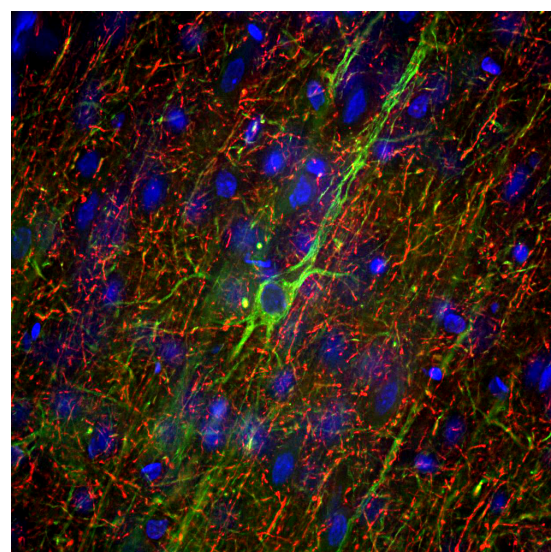


Immunofluorescence analysis of adult rat frontal cortex section stained with mouse mAb to neurofilament NF-M, MCA-3H11, dilution 1:5,000 in green, and costained with chicken pAb to neurofilament NF-H, CPCA-NF-H, dilution 1:5,000 in red. Following transcardial perfusion of rat with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45μM, and free-floating sections were stained with above antibodies. MCA-3H11 antibody labels neuron cell bodies and dendrites of pyramidal neurons, as well as dendrites and axons of other neuronal cells, while the NF-H antibody, which is primarily directed against the heavily phosphorylated axonal forms of this molecule, and so stains the network of neuronal axons only.

## Background:

Neurofilaments are the 10nm or intermediate filament proteins found specifically in neurons, and are composed predominantly of three major proteins called NF-L, NF-M and NF-H. NF-M is the neurofilament middle or medium molecular weight polypeptide and runs on SDS-PAGE gels at 145-160kDa, with some species variability, though the real molecular weight is ~105kDa. The major function of neurofilaments is likely to control the diameter of large axons (1). Antibodies to NF-M such as MCA-3H11 are useful for identifying neuronal cells and their processes in tissue sections and in cell culture. NF-M antibodies can also be useful to visualize neurofilament rich accumulations seen in many neurological diseases, such as Amyotrophic Lateral Sclerosis (a.k.a. Lou Gehrig's disease) and Alzheimer's disease (2-4). Much recent evidence has suggested that the detection of NF-L and NF-H in blood and CSF might be a useful prognostic or diagnostic biomarkers of neuronal damage and degeneration associated with a variety of CNS pathologies (5,6). The potential utility of NF-M in this fashion has not to date been examined.

The MCA-3H11 antibody was made against a recombinant fusion protein of E. coli TrpE fused to the C-terminus of rat NF-M, amino acids 677-845 (7). The epitope was mapped using protein cleavage to the extreme C-terminal sequence, amino acids 762-845, a region of very conserved protein sequence which includes some interesting sequence repeats of currently unknown function (8). This epitope corresponds to amino acids 833-916 of the slightly larger human NF-M sequence. The antibody works on a variety of species and is clean on western blots of crude lysates, on cells in culture and staining of sectioned material. The antibody has been widely used for many years, for example the original observation that the widely used HEK293 cell line has unexpected neuronal properties was made with this antibody (9). Also available from EnCor are rabbit and chicken polyclonal antibodies to the same immunogen RPCA-NF-M, and CPCA-NF-M. All three antibodies works on a variety of species and are clean and specific on western blots, cell and tissue staining.

**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 Co—Cow Pi—Pig Ho—Horse Ch—Chicken
Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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