NF-L DegenoTag™ Peptide Mouse Monoclonal Antibody

MCA-6H63

Ordering Information
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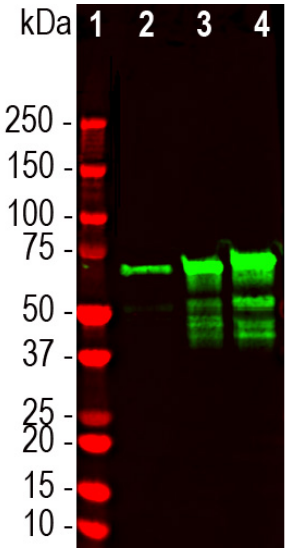
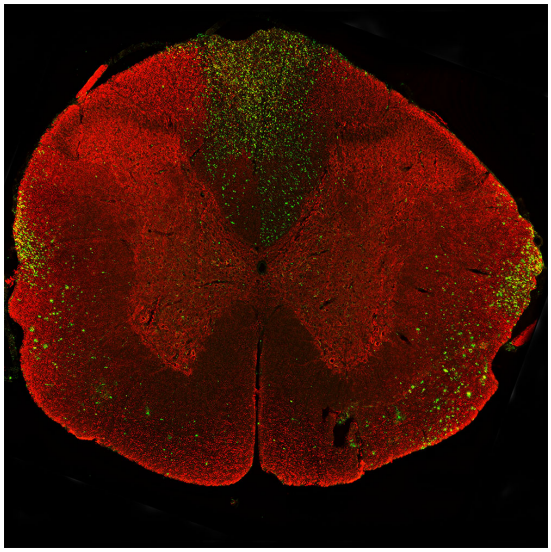
HGNC Name: *NEFL*
UniProt: *P07196*
RRID: *AB_2923484*
Immunogen: *Proprietary recombinant construct containing amino acids of human NF-L expressed in and purified from E. coli.*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Shipped on ice. Store at 4°C for short term, for longer term at -20°C. Avoid freeze / thaw cycles.*
Recommended dilutions:
WB: 1:5,000. ICC/IF: 1:10,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, ICC/IF, ELISA	Mouse	IgG1 heavy, κ light	68-70kDa by SDS-PAGE	Hu, Rt, Ms, Bo, Po



Western blotting of MCA-6H63 on crude CNS homogenates. Lane 1 shows molecular weight standards of indicated size. Lane 2 shows western blot of homogenate of E20 rat spinal cord, lane 3 homogenate of adult rat spinal cord and lane 4 shows homogenate of cow spinal cord extract. The MCA-6H63 antibody binds the denatured forms of NF-L with apparent molecular weight 68-70kDa as immobilized on western blotting membranes. Lower molecular weight bands under the major band are proteolytic fragments of NF-L.



Immunostaining of a coronal section of the spinal cord of a rat given a midline C4 contusion injury three days previously. Sections were stained with RPCA-NF-L-ct (red) and MCA-6H63 in green. MCA-6H63 stains prominent aggregates of material concentrated in the lateral funiculi and the dorsal columns but seen in lesser amounts throughout the section. These are degenerating and degenerated axons damaged by the C4 lesion. The RPCA-NF-L-ct antibody binds the C-terminal "tail" region of NF-L which is absent or destroyed during degeneration, so the MCA-6H63 positive profiles are largely negative for RPCA-NF-L-ct.

Background:

We have recently developed a series of novel antibody reagents which we call DegenoTag™ products. These are antibodies which recognize epitopes in a small segment of the neurofilament NF-L subunit which are normally not accessible to antibodies but which became available on degeneration. We have evidence that these epitopes are made accessible as a result of degeneration induced proteolysis, and in agreement with this hypothesis we could make previously negative control tissues become strongly DegenoTag™ antibody positive by treatment with proteases. In addition healthy CNS tissues do not stain with DegenoTag™ reagents except for a tiny minority of apparently spontaneously degenerating neuronal cells and processes. In stark contrast DegenoTag™ reagents strongly bind numerous profiles in tissues from animals given experimental spinal cord injuries. We also discovered that our antibodies to the C-terminal of NF-L, such as our rabbit polyclonal RPCA-NF-L-ct and mouse monoclonal MCA-DA2 fail to stain these degenerated profiles. Our reagents can therefore be used to positively identify both healthy and degenerated processes. Process and cells undergoing degeneration show both types of Degenotag™ reagent.

MCA-6H63 was raised against a proprietary recombinant immunogen based on the Coil 2 region of human NF-L. Further studies showed that the MCA-6H63 epitope is dependent on amino acids 305-316. The antibody works well on western blots of a variety of species but binds only degenerating or degenerated processes in sectioned material (7, 8). Other Uman type antibodies we market are MCA-1D44 and MCA-1B11. Full details of these findings are described in our a BioRχiv and in greater detail in a peer-reviewed publication in Brain Communications. It also works well on paraffin embedded histological sections of rodent CNS tissues, including transgenic mouse models. It is also an excellent capture reagent in ELISA. EnCor also markets other Degenotag™ reagents such chicken and rabbit polyclonals to the same NF-L region CPCA-NF-L-Degen and RPCA-NF-L-Degen which share this interesting degeneration specific staining pattern.

References:

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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. *Ayers J. L. et al. Prion-like propagation of mutant SOD1 misfolding and motor neuron disease spread along neuroanatomical pathways. Acta Neuropathol. 131:103-114 (2016).*
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8. *Shaw G, et al. Uman type neurofilament light antibodies are effective reagents for the imaging of neurodegeneration. Brain Communications doi.org/10.1093/braincomms/fcad067.*

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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