Neurofilament NF-H Goat Polyclonal Antibody

GPCA-NF-H

Ordering Information
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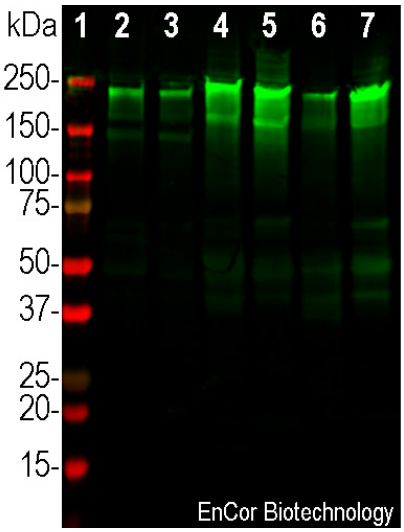
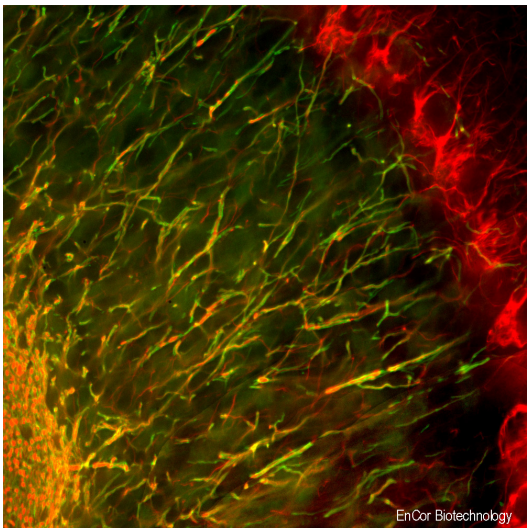
HGNC Name: NEFH
UniProt: P12036
RRID: AB_2923488
Immunogen: Native NF-H purified from bovine spinal cord
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3
Storage: Store at 4°C for short term, for longer term at -20°C.
Recommended dilutions:
WB: 1:10,000-25,000. ICC/IF and IHC: 1:1,000-5,000.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Goat		200-220kDa by SDS-PAGE	Hu, Rt, Ms, Co, Pi, Ho



Western blot analysis of tissue lysates from different species using goat pAb to NF-H, GPCA-NF-H, dilution 1:20,000 in green: [1] protein standard (red), [2] rat brain, [3] mouse brain, [4] cow cerebellum, [5] cow spinal cord, [6] pig hippocampus and [7] pig spinal cord. Strong band at about 220kDa corresponds to the major phospho-NF-H subunit. Smaller proteolytic fragments of NF-H are also detected in some preparations.



Immunofluorescence analysis of mouse cerebellum section stained with goat pAb to NF-H, GPCA-NF-H, dilution 1:3,000 in red, and costained with mouse mAb to myelin basic protein (MBP), MCA-7G7, dilution 1:5,000 in green. Following transcardial perfusion with 4% paraformaldehyde, mouse brain was post fixed for 24 hours, cut to 45µM, and free-floating sections were stained with above antibodies. The NF-H antibody labels axons of basket and Purkinje cells and others. The MBP antibody stains oligodendrocyte cell bodies and the myelin sheathes around axons in the granular layer at center and the white matter at bottom left.

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, though other proteins may also be present. NF-H is the neurofilament high or heavy molecular weight polypeptide and runs on SDS-PAGE gels at 200-220 kDa, with some variability across species boundaries. The protein is in reality much smaller in molecular size, about 110kDa (1,2). The unusual SDS-PAGE mobility is due partly to a very high content of charged amino acids, particularly glutamic acid rich regions, and the non-phosphorylated form runs on SDS-PAGE at about 160kDa. The predominant type of NF-H is the axonal form which is heavily serine phosphorylated on 40 or more tandemly repeated lysine-serine-proline (KSP) containing peptides (3-5). The phosphorylation of these peptides results in considerable further retardation on SDS-PAGE gels, so the heavily phosphorylated axonal form runs at 200-220kDa with some species variability. Antibodies to NF-H are useful for identifying axonal processes in tissue sections and in culture. NF-H antibodies can also be useful in visualizing neurofilament accumulations seen in many neurological disorders, such as Amyotrophic Lateral Sclerosis (also known as Lou Gehrig's disease), Alzheimer's disease and following traumatic injury. The phosphorylated axonal form of NF-H usually referred to as pNF-H, can be detected in blood and CSF following a variety of damage and disease states resulting in axonal compromise, and antibodies such as this can be used to used to quantify such ongoing axonal loss (e.g. 6-8). The GPCA-NF-H antibody was raised against biochemically isolated NF-H purified from bovine spinal cord (9). This preparation is dominated by axonal forms of NF-H which are heavily phosphorylated on the multiply repeated NF-H KSP type sequences, and this antibody reacts very strongly with these phosphorylated repeats. Reactivity with non-phosphorylated KSP sequences is orders of magnitude weaker, similar to other characterized antibodies to NF-H (5). In most species there is some cross-reactivity with the phosphorylated KSP sequences found in the related neurofilament subunit NF-M which are similar but not identical to those of NF-H. The antibody recognizes phosphorylated NF-H strongly in all mammals tested to date and also in chicken. RPCA-NF-H recognizes neurofilaments in frozen sections, in tissue culture and in formalin fixed sections. We also supply three mouse monoclonal antibodies a widely used chicken and rabbit polyclonal antibodies made to the same immunogen, MCA-NAP4, MCA-9B12, MCA-AH1, CPCA-NF-H, and RPCA-NF-H.

References:

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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 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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