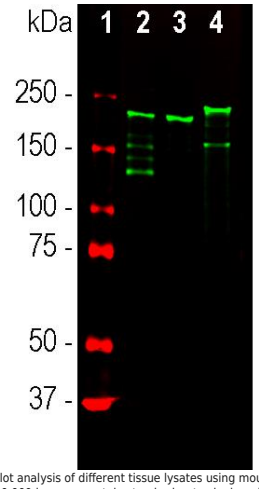
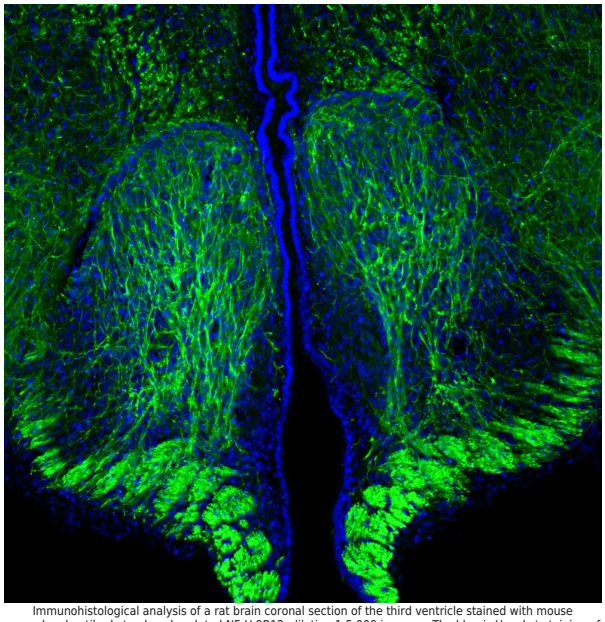
Product name:	Neurofilament Heavy (NF-H)
Cat number:	MAB-94403
Conjugate:	Unconjugated
Size:	100 ug
Clone:	9B12
Concentration:	1mg/ml
Host:	Ms
Isotype:	IgG2b
Immunogen:	Native NF-H purified from bovine spinal cord, binding to phosphorylated KSP sequences
Reactivity:	Hu Rt Ms,Cw, Pg
Applications:	Western Blot: 1:10,000 Immunocytochemistry: 1:1,000 Immunofluorescence: 1:1,000 Immunohistochemistry: 1:1,000
Molecular Weight:	200-220 kDa
Purification:	Purified
Form:	Liquid
Buffer:	Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN3
Storage:	Store at 4°C. For long term storage, leave frozen at -20°C. Avoid freeze / thaw cycles

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 160-220 kDa, with some variability across species boundaries though in reality is much smaller, about 110kDa (1,2). The unusual SDS-PAGE mobility is due to a very high content of negatively charged amino acids 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. The phosphorylation of these peptides results in 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 neurological disorders, such as amyotrophic lateral sclerosis, 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. 9B12 is a mouse monoclonal antibody raised against native axonal phosphorylated NF-H purified from bovine spinal cord. 9B12 recognizes the phosphorylated NF-H KSP sequences similar to other antibodies to NF-H . There is some cross-reactivity with the phosphorylated KSP sequences found in the related neurofilament subunit NF-M. The antibody recognizes NF-H strongly in all mammals tested to date and also in chicken. It recognizes neurofilaments in frozen sections in tissue culture and in formalin fixed sections.



Western blot analysis of different tissue lysates using mouse mAb to NF-H, 9B12, dilution 1:10,000 in green:protein standard, rat spinal cord mouse spinal cord, and cow spinal cord. Strong band at about 200-220kDa corresponds to the major phosphorylated form of the NF-H subunit. Smaller proteolytic fragments of NF-H are also detected in some preparations.



Immunohistological analysis of a rat brain coronal section of the third ventricle stained with mouse monoclonal antibody to phosphorylated NF-H,9B12, dilution 1:5,000 in green. The blue is Hoechst staining of nuclear DNA. Following transcardial perfusion with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45µM, and free-floating sections were stained with above antibody. The 9B12 antibody is a robust marker of the axons of neuronal cells.