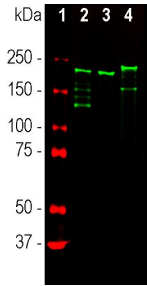


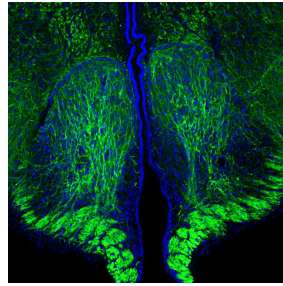
Cat. No:	MAB-94403
Conjugate:	Unconjugated
Size:	100 ug
Clone:	9B12
Concentration:	1mg/ml
Host:	Ms
Isotype:	IgG2b
Immunogen:	Native NF-H purified from bovine spinal cord, binding to phosphorylated KSP sequences
Reactivity:	Hu Rt Ms,Cw, Pg
Applications:	Western Blot: 1:10,000 Immunocytochemistry: 1:1,000 Immunofluorescence: 1:1,000 Immunohistochemistry: 1:1,000
Molecular Weight:	200-220 kDa
Purification:	Purified

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 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.

Form:	Liquid
Buffer:	Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM Na ₃
Storage:	Store at 4°C. For long term storage, leave frozen at -20°C. Avoid freeze / thaw cycles



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.

The blue is Hoechst staining of nuclear DNA.

Following transcardial perfusion with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45 μ M, and free-floating sections were stained with above antibody.

The 9B12 antibody is a robust marker of the axons of neuronal cells.