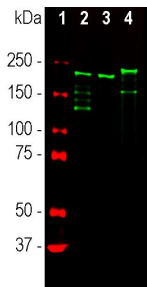


|                          |  |
|--------------------------|--|
| <b>Cat. No:</b>          | MAB-94403  |
| <b>Conjugate:</b>        | Unconjugated   |
| <b>Size:</b>             | 100 ug   |
| <b>Clone:</b>            | 9B12   |
| <b>Concentration:</b>    | 1mg/ml   |
| <b>Host:</b>             | Ms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

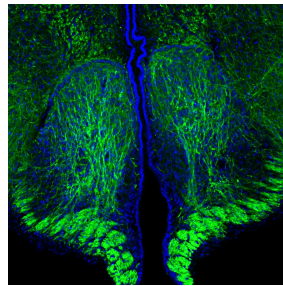
**Background:**

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.

|                 |  |
|-----------------|--|
| <b>Form:</b>    | Liquid   |
| <b>Buffer:</b>  | Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM Na <sub>3</sub> |
| <b>Storage:</b> | 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.

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

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

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