Technical Data Sheet

Purified Mouse Anti-Neurofilament Protein (NF-M) w/Control

Product Information

Material Number:	**551962**
Size:	50 µg
Reactivity:	QC Testing: Rat Reported: Human, Rabbit, Dog, Chicken, Mouse, Hamster, Cow, Monkey, Guinea Pig, Sheep, Xenopus
Component:	**51-8124KC**
Description:	Purified Mouse Anti-Neurofilament Protein (NF-M)
Size:	50 µg (1 ea)
Concentration:	0.5 mg/ml
Clone Name:	RNF406
Immunogen:	Neurofilament cytoskeletal preparation from calf brain
Isotype:	Mouse IgG1
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.
Component:	**51-16646N**
Description:	Rat Whole Brain Lysate
Size:	50 µg (1 ea)
Concentration:	1.0 mg/ml
Storage Buffer:	SDS-PAGE buffer (62mM Tris pH 6.8, 2% SDS, 0.9% b-mercaptoethanol, 0.003% bromophenol blue, 5% glycerol)

Description

Intermediate filaments (IF) are a subset of cytoskeletal proteins which function to give overall structural integrity to the plasma membrane as well as organize cells 160kDa into specific tissues. IF proteins can be divided into six major types based upon the similarity in sequence. Neurofilaments (NF) are classified as Type IV intermediate filaments and are composed of three polypeptides, designated NF-L (~68 kDa), NF-M (~160 kDa), and NF-H (~200 kDa) which differ in molecular weight. The distribution of these neurofilaments is mostly limited to the central and peripheral nervous systems and restricted to neurons. NF proteins function to provide radial growth of the neuron. Most neurons are composed of all three NF proteins, although the role of each individual NF polypeptide has not been fully elucidated. Both phosphorylated and non-phosphorylated forms of NF's are found in the brain; phosphorylation status is dependent upon the stage of development and region of the brain. The exact role for the phosphorylation of neutrofilaments remains to be elucidated, but aberrant neurofilament phosphorylation occurs in a number of neurodegenerative diseases. For example, in a rat model for spontaneous type I diabetes, the NF-M neurofilament in the sural nerve of BB rats showed a 2.5-fold increase in phosphorylation. Phosphorylation may play a role in regulating the incorporation of slow transported neurofilament proteins into the stable cytoskeletal network of the axon, thereby helping to regulate the diameter of the axon. The antibody only recognizes the phosphorylated form of neurofilament NF-M; it does not recognize the nonphosphorylated form of the molecule. A neurofilament cytoskeletal preparation from calf brain was used as the immunogen. The antibody has only been evaluated in rat, but may also recognize NF-M from other species due to the highly conserved nature of this molecule.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store the antibody at 4°C. Store the positive control lysate (Cat. No. 51-16646N) at -20°C.

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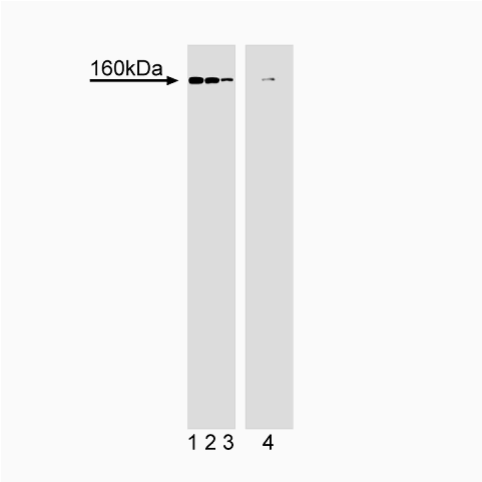

Western blot analysis of NF-M. *Lysate from rat whole brain was either untreated (lanes 1-3) or treated with alkaline phosphatase (50 µg/ml at 37°C for 30 minutes, lane 4). The lysate was then probed with anti-neurofilament (clone RNF406, Cat. No. 8124KC) at concentrations of 1.0 (lane 1), 0.5 (lanes 2, 4), and 0.25 µg/ml (lane 3). RNF406 is identified as a band of 160 kDa. Alkaline phosphatase treatment caused a significant reduction of the NF-M band.*

Application Notes

Application

Western blot	Routinely Tested

Recommended Assay Procedure:

Applications include western blot analysis (0.25-1.0 µg/ml). Rat whole brain lysate [50 µg (1 µg/µl)] is provided as a ready-to-use western blot positive control (Cat. No. 51-16646N).

It has been reported, but not tested at BD Pharmingen, that clone RNF406 cross-reacts with human, rabbit, dog, chicken, mouse, hamster, cow, monkey, guinea pig, sheep and xenopus.

BD Biosciences Pharmingen offers several neurofilament antibodies. Lysate from rat whole brain was used to evaluate these antibodies; these results are summarized in the following table. However, actual bands observed could vary according to the cell model system used.

Clone	Catalog Number	Phospho-specific	MW (kDa)	Isotype
RNF402	8100KK/551349	No	200	Mouse IgM
RNF403	8122HN/551957	Yes	160	Mouse IgG^{1}
RNF404	8098KK/551348	Yes	200	Mouse IgG^{2a}
RNF405	8123HN/551958	Yes	200	Mouse IgM
RNF406	8124HN/551962	Yes	160	Mouse IgG^{1}

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Fernyhough P, Gallagher A, Averill SA, et al. Aberrant neurofilament phosphorylation in sensory neurons of rats with diabetic neuropathy. *Diabetes*. 1999; 48(4):881-889.(Biology)

Kuijpers W, Tonnaer EL, Peters TA, Ramaekers FC. Expression of intermediate filament proteins in the mature inner ear of the rat and guinea pig. *Hear Res*. 1991; 52(1):133-146.(Biology)