**Introduction:**

In the nervous system, two major types of intermediate filaments are recognised: neurofilaments (NFs; type IV neuron-specific intermediate filaments) and glial filaments. NFs exist as 10nm filaments in the axoplasm of mature neurons, where they function by giving form and tensile strength to the cell processes, i.e. dendrites and axons. Mammalian neurofilaments are composed of three immunochemically distinct polypeptides with predicted molecular masses of 62 (NF-L), 102 (NF-M) and 110 (NF-H) kDa, respectively. The relative mobilities of human NF subunits as determined by SDS-PAGE are considerably higher than the predicted values at approximately 68/70kDa (NF-L), 150kDa (NF-M) and 200/210kDa (NF-H), due almost entirely to phosphorylation of the subunits. In keeping with other intermediate filaments, neurofilaments are highly resistant to extraction at physiological pH and exhibit a high degree of helicity.

The neurofilament proteins are encoded by separate genes, and their co-expression in neuronal perikarya is well reported, however, due to microheterogeneity among NF proteins conferred by phosphorylation, proteolysis and conformational rearrangements of the filament structure, the failure to detect NF subunits in a population of neurons may reflect methodological limitations rather than the real absence of the antigens.

**Product information:**

Primate neurofilament proteins were obtained and highly purified by high pressure liquid chromatography, prior to immunisation. The NA 1211 antiserum is reactive with neurofilament H (200/220kDa) having a high degree of phosphorylation. On two-dimensional immunoblots, the antiserum yields a single band at the non-phosphorylated NF-H position at 200kDa, pK 5.1.

**Application data**

This antiserum stains thick and thin axons and also yields faint staining of occasional cell bodies. It reveals a specially rich network of thin axons. Antiserum NA 1211 does not react with non-neuronal tissue components. It reacts strongly with rat, bovine and human neurofilaments.

The antiserum may be used in Western blotting and immunocytochemical applications at dilutions of 1:500 or greater when used in conjunction with sensitive detection methods. Optimal dilutions must be determined by experimentation. The antiserum may be used successfully on (para-) formaldehyde-fixed cryostat, Vibratome and de-paraffinised tissue sections, but the use of glutaraldehyde alone as a primary fixative is not recommended. In ELISA tests, the antiserum shows 100% reactivity with bovine NF-H, less than 1% cross-reactivity with bovine NF-L and NF-M.

*Such as enhanced chemiluminescence (ECL; Amersham) for Western blotting applications, and ABC-Elite (Vector Laboratories) for immunohistochemistry. Antigen retrieval techniques may enhance immunostaining intensity, but users are advised to carry out appropriate control experiments.

**Vial contents, Storage and Use:**

See vial label for volume. Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes prior to freezing if appropriate (see overleaf). The antiserum has not been purified. Dilute to working strength with 50mM Tris-HCl buffer (pH 7.6) containing 1.5% sodium chloride and 1% normal goat serum (if a goat anti-rabbit IgG linker antibody is to be used). Store diluted antibody at 2-4°C and use within 1 month.
## REFERENCES