Neurofilament light (NfL)

Neurofilaments are the main cytoskeletal structure proteins in neurons (1). They are approximately 10 nm in diameter, which makes them thicker than actin and thinner than myosin. Therefore, they are classed as intermediate filaments (IFs) along with filaments built of keratins, nuclear lamins, and other members of the IF protein family. Neurofilaments comprise four different subunits, the stoichiometry of which varies depending on the maturity of the neuron. Three of these subunits, NfL (light), NfM (middle), and NfH (heavy), are type IV IF proteins and they are always present. Meanwhile, the fourth subunit is either α-internexin (also type IV) or peripherin (type III), for the central and peripheral nervous systems, respectively (see Figure 1). The NfM and NfH, which have long and highly charged C-terminal domains, are mostly found on the outside of the filaments whereas NfL, along with α-internexin or peripherin, forms the filament backbone (2). Thus, NfL plays a crucial role for the neurofilament polymerization process as well as for axonal structure maintenance.

Biochemical properties of NfL

All NFs have a variable N-terminal globular head, a conserved alpha-helical rod domain that carries hydrophobic repeats facilitating coiled-coil formation, and a C-terminal tail domain. NFs undergo post-translational modifications, phosphorylation, and glycosylation on their head and tail regions (3). These modifications contribute to the assembly, structure, and functions of NFs (2). The head domain of human NfL contains serine and threonine residues that undergo phosphorylation, as well as consensus sites for O-linked glycosylation. Whereas the tail domains of NfM and NfH contain abundant glutamic acid-rich and lysine-rich stretches of variable length with multiple serine phosphorylation sites, NfL has a much shorter C-terminal tail and is thus much less extensively phosphorylated. Human NfL consists of 543 amino acid residues, with an overall theoretical pI of 4.63 and a molecular mass of 61.4 Da. NfL can form homopolymers *in vitro* (2). A schematic presentation of neurofilament assembly *in vivo* is presented in Figure 2.

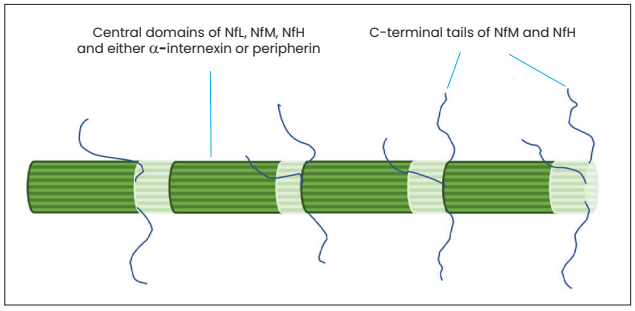


Figure 1.
Subunits of neurofilaments.

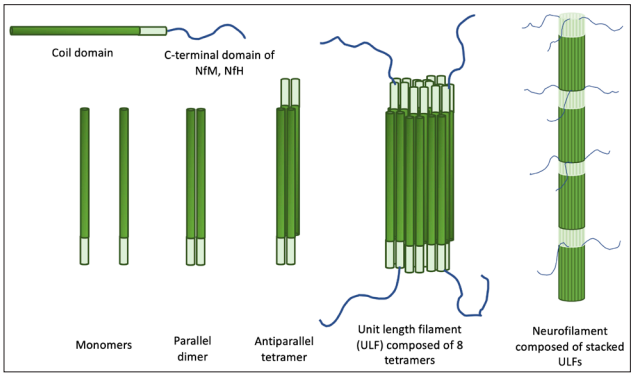


Figure 2.
Simplified schematical view of neurofilament assembly.

CLINICAL UTILITY
- **Traumatic brain injury**
- **Neurodegenerative disease**

NfL as a diagnostic marker of neuronal damage

Axonal injury and neurodegeneration lead to the appearance of neuronal proteins in the cerebrospinal fluid (CSF). Given that NFs are abundant proteins that are exclusively expressed in neurons, they may serve as a marker of neuronal degradation (4). Numerous studies have indicated that NFs can be markers of acute conditions (stroke or trauma), as well as a variety of neurological diseases such as Alzheimer's disease, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), frontotemporal dementia, diabetic neuropathy, and other pathological processes accompanying central or peripheral neuropathies (5). Monitoring NfL levels in CSF or blood is useful in clinical diagnostic evaluations for predicting the progression of various acute and chronic neurological diseases, as well as for assessing treatment efficacy (4).

Reagents for developing a reliable NfL assay

Hytest offers several monoclonal antibodies (MAbs) for the development of a sensitive and specific NfL immunoassay. MAbs recognize NfL in CSF with high specificity and sensitivity. Our antibodies do not have cross-reactivity to type III IF proteins (GFAP, vimentin, desmin, peripherin) and several other neuronal proteins. Pairs of MAbs effectively recognize both recombinant and endogenous NfL, and they may be used for a variety of immunoassays, such as direct EIA, indirect EIA, and sandwich-type immunoassays. Hytest's anti-NfL antibodies are a good choice for the development of highly sensitive immunoassays such as chemiluminescent (CLIA), single-molecule immunoassays (SIMOA), and others.

Monoclonal antibodies specific to human NfL

We provide several monoclonal antibodies specific to human NfL that are derived from three different species and are designed to be used in NfL-specific immunoassays. The basic information relating to our antibodies is summarized in Table 1.

All MAbs effectively detect NfL in a variety of applications. Out of the five possible MAb pairs listed in Table 2, we recommend foremost the pairs NF79-NF71, NF79-NF36, and NF36-NF71. However, as assay performance is dependent on the platform, label, buffer, assay conditions, etc., the incorporation of additional capture or detection antibodies to make 2+1, or 1+2 combinations can also be recommended for improved assay performance.

Detection antibodies may be labelled with HRP, ALP, biotin, or other labels. Figures 3 and 4 show examples of calibration curves for the prototype assay NF79-NF71, colorimetric and CLIA, respectively. The assay level of detection (LoD) in the best prototype sandwich-type immunoassay was 30 pg/ml for the colorimetric version and 10 pg/ml for the CLIA version, respectively. Assay sensitivity for Hytest's antibodies can be raised significantly by using highly sensitive platforms such as SIMOA and others.

Table 1.
General information on Hytest's anti-NfL MAbs

MAb	Species of Origin	Isotype
NF31	Mouse	IgG2b
NF36	Rabbit	IgG
NF71	Mouse	IgG2b
NF79	Rat	IgG2b

Table 2.
Recommended pair combinations for NfL sandwich immunoassays, preferred pairs marked in green.

Capture \ Detection	NF31	NF36	NF71
NF36	+		+
NF71	+		
NF79		+	+

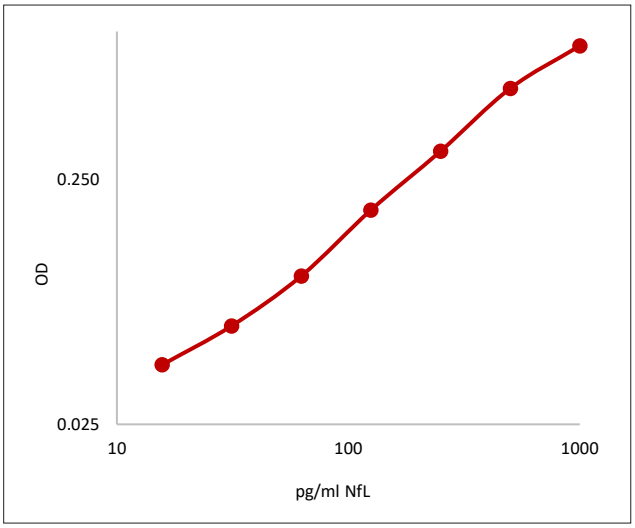


Figure 3.
Calibration curve for the prototype assay NF79-NF71. 3-step sandwich immunoassay. Detection antibodies were coated on Costar EIA/RIA plates. Purified endogenous bovine NfL (Uman Diagnostics) was used as a calibrator. Detection MAbs were labelled with biotin. Streptavidin-HRP conjugate was used for immune complex revealing.

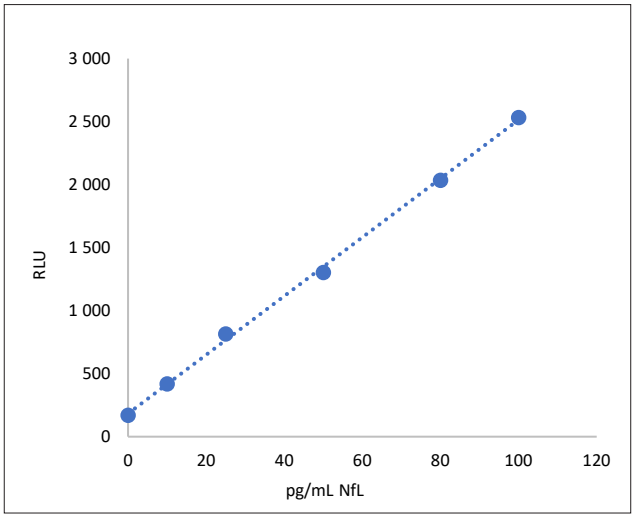


Figure 4.
Calibration curve for the CLIA assay prototype NF79-NF71. 1-step sandwich immunoassay. Capture MAbs were coated on streptavidin magnetic beads. Internal recombinant NfL (Hytest) was used as a calibrator. Detection MAbs were labelled with acridinium ester. Immune complexes were revealed by chemiluminescence measurement.

Specificity of anti-NfL assay prototypes

Excess amounts of type III IF proteins and several other neuronal proteins were applied to sandwich-type immunoassay prototypes. The in-house assays did not demonstrate any cross-reactivity to type III IF proteins or other tested neuronal proteins. Examples are shown in Figure 5.

Detection of NfL in clinical samples

Assays based on our MAb pairs can be used for the measurement of endogenous NfL in CSF samples. Figure 6 shows a dilution curve of NfL in CSF that was measured by the prototype ELISA assay NF79-NF71. The prototype assay NF79-NF71 was also used for the immunodetection of NfL in CSF samples from several patients with different diagnoses. Depending on the diagnosis, NfL levels were low, moderate, or high, as presented in Figure 7.

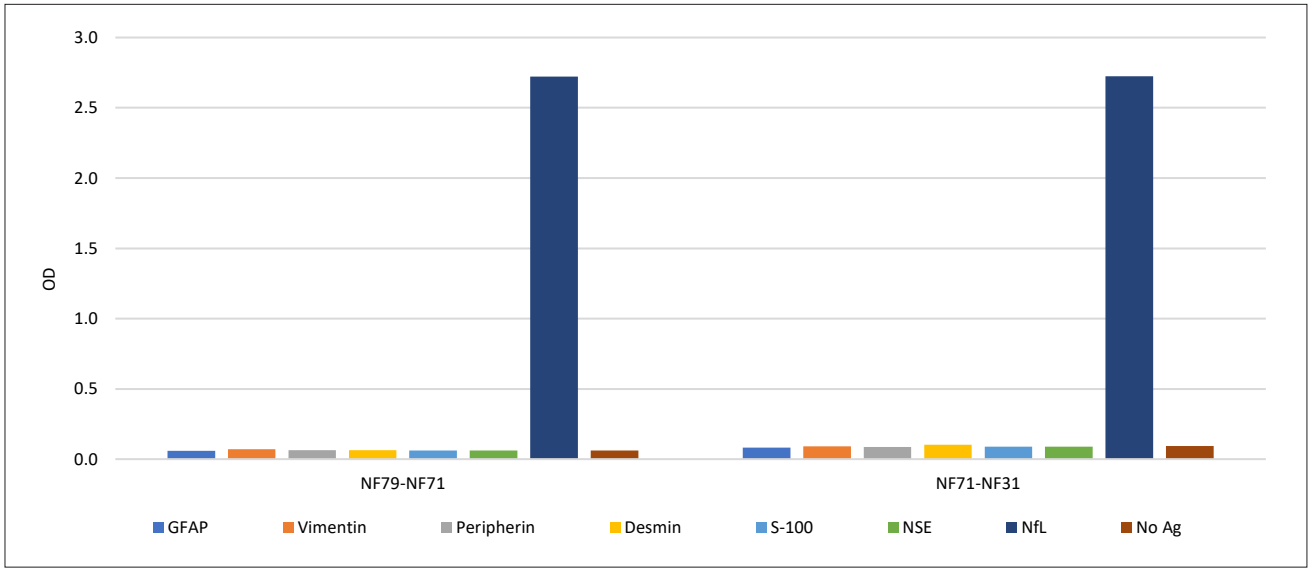


Figure 5.

Cross-reactivity of different prototype assays to various neuronal proteins. 3-step sandwich-type immunoassay. Recombinant proteins were added at a concentration of 1,000 ng/ml with recombinant NfL as a positive control.

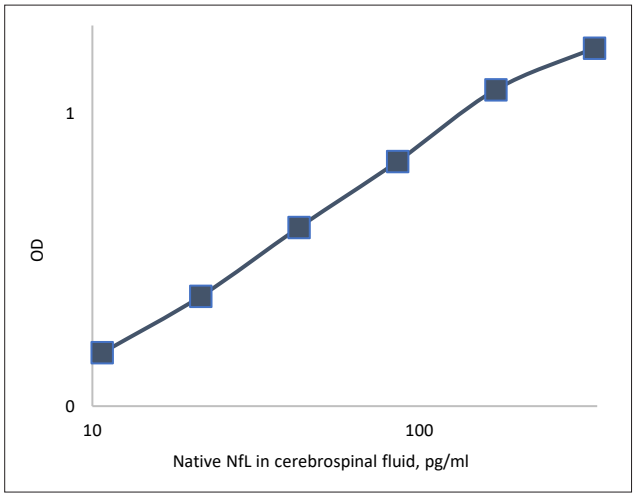
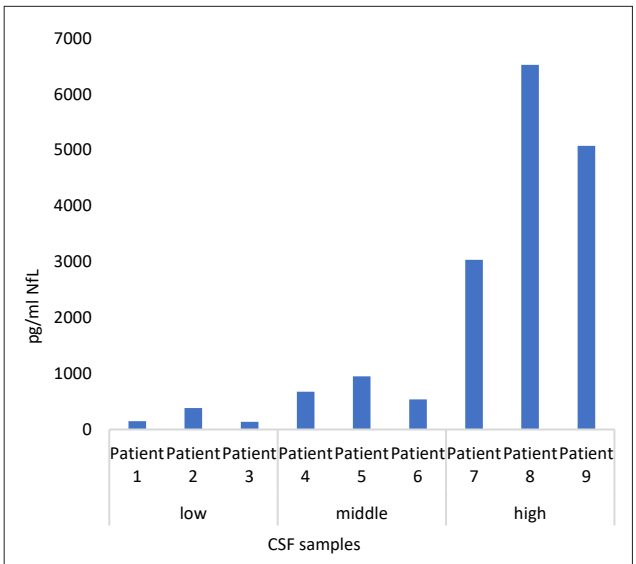


Figure 6.

Titration curve of endogenous NfL in a CSF sample. The titration curve was measured using the NF79-NF71 prototype in-house sandwich immunoassay. The concentration of NfL in the CSF sample was initially measured by the Uman Diagnostics NF-light® ELISA assay.



Patient 1	Migraine
Patient 2	Concussion
Patient 3	Pneumonia
Patient 4	Chronic cerebral ischemia
Patient 5	Unspecified stroke
Patient 6	Non-infectious encephalitis
Patient 7	Subarachnoid hemorrage
Patient 8	Dyscirculatory encephalopathy
Patient 9	Guillan-Barré syndrome

Figure 7.

NfL immunodetection in CSF samples. Prototype 3-step in-house sandwich-immunoassay NF79-NF71. The adjacent table lists the diagnosis of each patient. CSF samples were diluted 4-5-fold prior to measurement.

Prototype assays demonstrate a good correlation with a commercial assay. In 23 CSF samples from patients suffering from a variety of conditions, NfL levels were measured by the commercially available NF-Light® ELISA (Uman Diagnostics), as well as by three prototype assays (NF79-NF71, NF79-NF36 and NF36-NF71). The colorimetric prototype assays and the commercial assay are both 3-step sandwich-type ELISAs with a biotinylated detection MAb. As shown in Figure 8, all of Hytest's prototype assays show good linear correlations with the reference assay.

Anti-NfL MAb NF36 can also be used for the immunochemical staining of NfL. An example of such staining is shown in Figure 9.

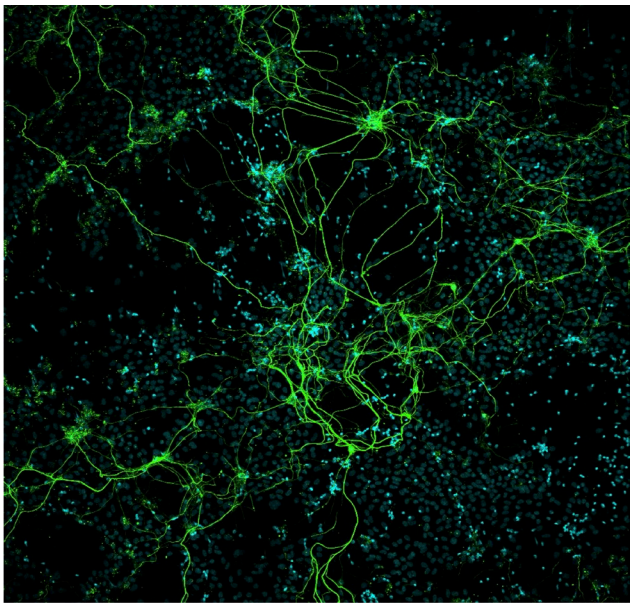


Figure 9.
Immunochemical staining of NfL in cultivated murine neurons. Neurons were isolated from the hippocampus of mouse embryo. The primary antibody was NF36, while the secondary antibody was anti-rabbit polyclonal antibodies conjugated with Alexa-488 (green). Nuclei were stained with Hoechst 33342.

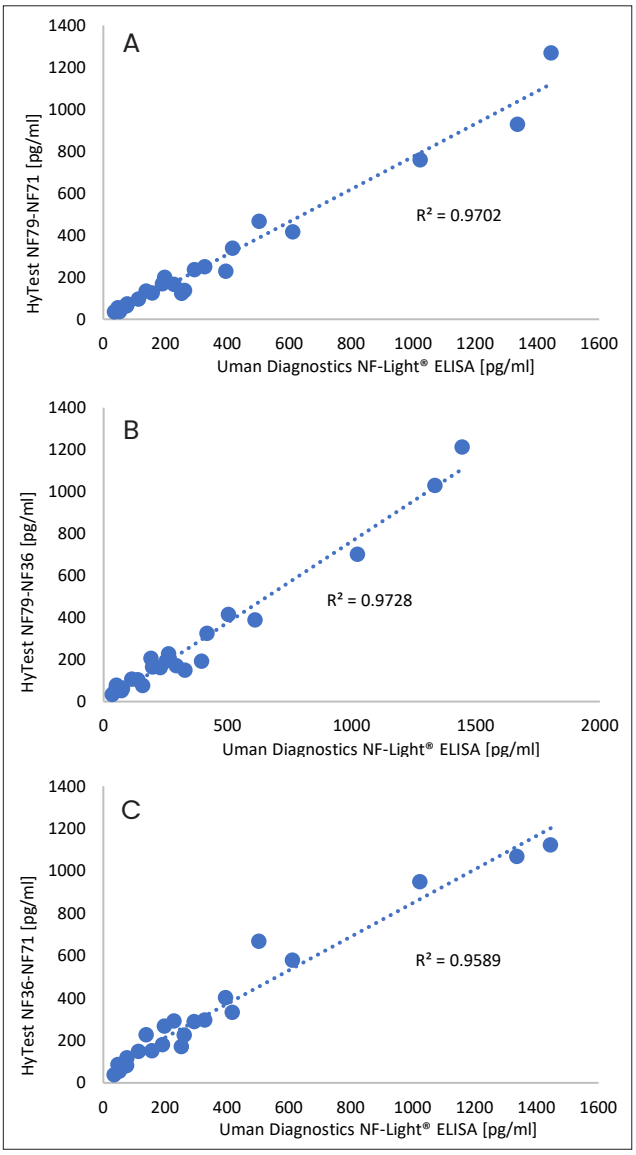


Figure 8.
Correlation between measurement results of NfL in 23 CSF samples with the NF-Light® ELISA (Uman Diagnostics) and three prototype ELISA assays with Hytest's antibodies: A) NF79-NF71, B) NF79-NF36, and C) NF36-NF71.

REFERENCES

1. **Lee MK, Cleveland DW.** Neuronal intermediate filaments. Annual Revew of Neuroscience. 1996, 19 pp.187-217.
2. **Yuan A et al.** Neurofilaments and Neurofilament Proteins in Health and Disease. Cold Spring Harbor Perspectives in Biology. 2017, Apr 3; 9(4): a018309.
3. **Grant P, Pant HC.** Neurofilament protein synthesis and phosphorylation. Journal of Neurocytology. 2000, 29(11-12) pp. 843-72.
4. **Khali M et al.** Neurofilaments as biomarkers in neurological disorders. Nature Review Neurology. 2018, Oct; 14(10) pp. 577-589.
5. **Braissant O.** Neurofilament Proteins in Brain Diseases. New Research on Neurofilament Proteins. 2007, pp. 25-51.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Neurofilament light (NfL), human	4NF3	NF31	IgG2b	EIA
		NF71	IgG2b	EIA
		NF36	IgG	EIA, IHC, recombinant rabbit antibody
		NF79	IgG2b	EIA, rat monoclonal antibody

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • HYTEST.FI