Neurofilament light (NfL)

Neurofilament light chain (NfL) is a neuron-specific protein expressed in both the central and peripheral nervous systems. It belongs to the intermediate filament (IF) protein family. Neurofilaments (NFs), a class of IFs, are major structural components of the neuronal cytoskeleton. They form the structural backbone of axons and play essential roles in maintaining axonal structure, growth and stability (1).

Like other IF proteins, neurofilament subunits share a common molecular organization consisting of a globular N-terminal head domain, a central α-helical coiled-coil rod domain and a variable C-terminal tail domain. The rod domain comprises coils 1A, 1B and 2, separated by linker regions (Figure 1).

Neurofilaments are assembled from four different subunits, whose relative proportions vary according to neuronal maturity. Three of these subunits, NfL, neurofilament medium chain (NfM), and neurofilament heavy chain (NfH), are consistently present, whereas the fourth is generally α-internexin in the central nervous system or peripherin in the peripheral nervous system. NfL, together with α-internexin or peripherin, forms the filament backbone. It therefore plays a crucial role in neurofilament assembly and the maintenance of axonal structure (2) (Figure 2).

IF proteins undergo extensive co-translational and post-translational modifications, including O-linked glycosylation, nitration, oxidation, ubiquitylation and phosphorylation. These modifications contribute to neurofilament assembly, structure and function (3). The head domain contains serine and threonine residues that can undergo phosphorylation and O-linked glycosylation. The tail domains of NfM and NfH contain abundant glutamic acid- and lysine-rich stretches of variable length with multiple serine phosphorylation sites. In contrast, NfL has a considerably shorter C-terminal tail and is therefore less extensively phosphorylated than NfM and NfH.

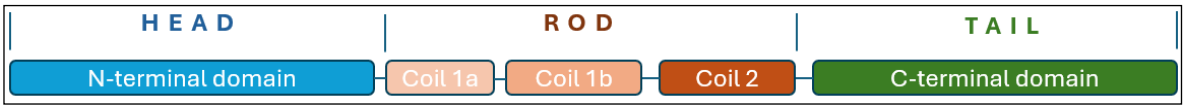


Figure 1.
Schematic representation of IF protein structure.

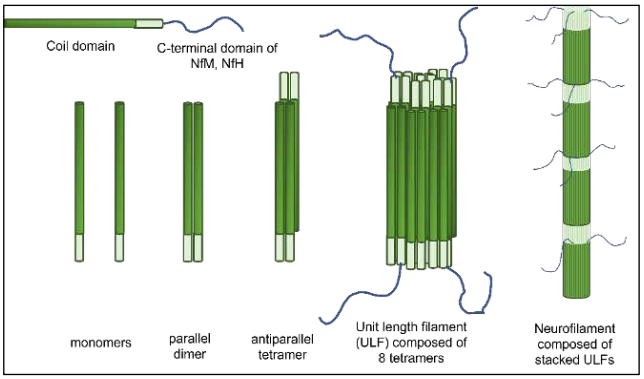


Figure 2.
Simplified schematic view of neurofilament assembly.

CLINICAL UTILITY

- **Traumatic brain injury**
- **Neurodegenerative disease**

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Human NfL (UniProt accession P07196) consists of 543 amino acid residues, with a theoretical isoelectric point of 4.63 and a calculated molecular mass of 61.5 kDa. Its apparent molecular mass determined by polyacrylamide gel electrophoresis may be higher than the calculated value because of its abundance of negatively charged amino acid residues and post-translational modifications, particularly phosphorylation and glycosylation.

NFL AS A MARKER OF NEURONAL DEGRADATION

Axonal injury and neuronal damage in the central or peripheral nervous system lead to the release of neuron-specific proteins into the cerebrospinal fluid (CSF) and blood. NFs, particularly NfL, can therefore serve as biomarkers of neuronal damage in acute conditions, such as stroke and traumatic injury, as well as in neurological diseases, including Alzheimer's disease, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), frontotemporal dementia and diabetic neuropathy (4).

NfL concentrations in CSF and blood vary widely depending on the extent of damage to large, myelinated axons and the rate of disease progression or activity. Monitoring NfL levels may support clinical evaluation by providing information about disease activity and progression and by helping assess treatment response in various acute and chronic neurological conditions (5).

Serial measurement of NfL, particularly when combined with disease-specific biomarkers, may be useful for monitoring ongoing neurodegeneration and evaluating recovery or treatment response.

REAGENTS FOR DEVELOPING RELIABLE NFL ASSAYS

Hytest offers a range of monoclonal antibodies (MAbs) and a recombinant NfL antigen for developing sensitive and specific NfL immunoassays. MAbs recognize native NfL in CSF with high specificity and sensitivity. They show no cross-reactivity with type III IF proteins (GFAP, vimentin, desmin, peripherin) and several other neuronal proteins. Recommended MAb pairs effectively recognize both recombinant and endogenous NfL and are suitable for various applications, including direct and indirect enzyme immunoassay (EIA), sandwich immunoassays, Western blotting (WB), immunocytochemistry (ICC) and immunohistochemistry (IHC). They are also well suited for developing highly sensitive assays using technologies such as chemiluminescent immunoassay (CLIA) and single-molecule array (Simoa®). Hytest's recombinant human NfL antigen is a highly pure and stable, full-length protein suitable for use in a wide range of immunoassays.

Recombinant human NfL

Hytest recombinant human NfL is produced in *E. coli* to support NfL immunoassay development and the manufacture of calibration and quality control reagents. A DNA fragment encoding full-length human NfL (543 amino acid residues) was used for expression.

The recombinant NfL was purified by ion-exchange chromatography and lyophilized from a buffer containing 16 mM Tris-HCl (pH 8.0), 0.8 mM EGTA, 4% sucrose and 2% SDS. The lyophilized format facilitates convenient handling and storage. The recombinant NfL antigen maintains consistent performance following repeated freeze–thaw cycles.

Its purity was evaluated by densitometric analysis following SDS-PAGE under reducing conditions and determined to be ≥95% (Figure 3). The main band (Band 1), with an apparent molecular mass of approximately 65 kDa, corresponds to full-length NfL. The additional band (Band 2), with an apparent molecular mass of approximately 28 kDa, corresponds to an NfL fragment comprising amino acid residues 354–543, as confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). The band (Band 3) with a mass at about 90 kDa corresponds to the full-length NFL as confirmed by MALDI-TOF LC MS/MS analysis, and presumably it represents the complex of full length and a fragment of NFL that was not disrupted under reducing conditions.

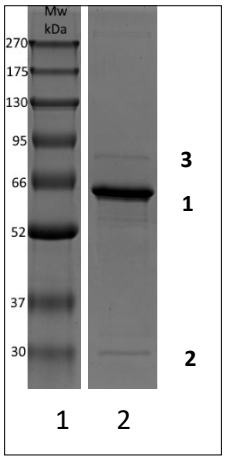


Figure 3.
SDS-PAGE analysis of recombinant NfL under reducing conditions using a 12.5% polyacrylamide gel. Lane 1, protein standards; lane 2, 2 µg of recombinant NfL. Band 1, full-length NfL; band 2, an NfL fragment corresponding to amino acid residues 354–543; band 3, a complex of full-length NfL and the NfL fragment.

NfL can be detected by WB using Hytest MAbs (Cat. #4NF3; Figure 4). Clones NF31, NF71 and NF79 are recommended for the detection of NfL by WB.

Monoclonal antibodies specific to human NfL

Hytest provides several MAbs specific to human NfL. They are derived from three species and are designed to be used in NfL-specific immunoassays. The basic information about the MAbs is summarized in Table 1 (page 3).

Applications of anti-NfL antibodies

All MAbs effectively detect NfL in a variety of applications. Out of the five possible MAb pairs listed in Table 2 (page 3), we

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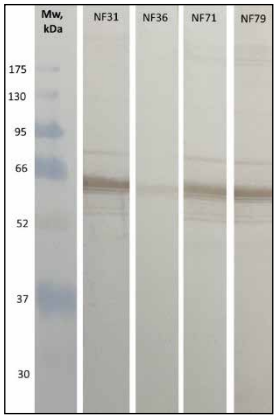


Figure 4.
Staining of recombinant NfL samples using Hytest NfL MAbs. SDS-PAGE (12.5% polyacrylamide gel) was performed under reducing conditions. The protein load was 0.5 µg of recombinant NfL per lane. Each antibody was applied at a concentration of 2 µg/mL. Lane 1, protein standards; lanes 2–5, recombinant NfL.

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., incorporation of additional capture or detection antibodies to make 2+1, 1+2 combinations can also be recommended for improved assay performance.

Detection antibodies may be labelled with HRP, ALP, biotin, or other suitable labels. Figure 5 shows representative calibration curves for the prototype CLIA using NF79 and NF71 as a sandwich antibody pair. The best-performing prototype sandwich immunoassay achieved a limit of detection (LoD) of 5 pg/mL. Assay sensitivity can be further improved by using Hytest antibodies on highly sensitive platforms such as Simoa® and other ultrasensitive technologies.

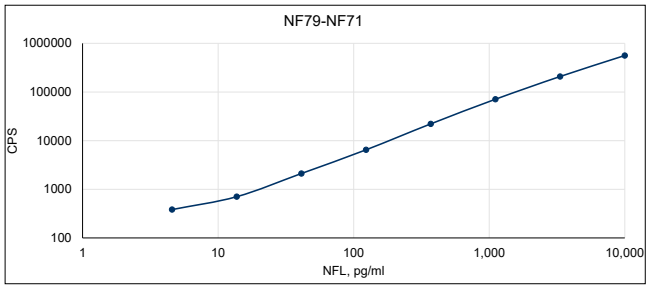


Figure 5.
Calibration curve for the prototype NF79–NF71 assay using a three-step sandwich immunoassay format. Capture MAb NF79 was coated onto 96-well CLIA plates. Recombinant human NfL (Cat. # 8NF5) was used as the calibrator. Detection MAb NF71 was biotinylated, and a streptavidin–poly-HRP conjugate was used for signal generation.

Specificity of anti-NfL assay prototypes

Type III IF proteins and several other neuronal proteins were tested in prototype sandwich immunoassay to evaluate the cross-reactivity of anti-NfL antibodies. The in-house assays showed no cross-reactivity with any of the tested proteins, even when they were added at concentrations of up to 1,000 ng/mL. Representative results are shown in Figure 6.

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		+	+

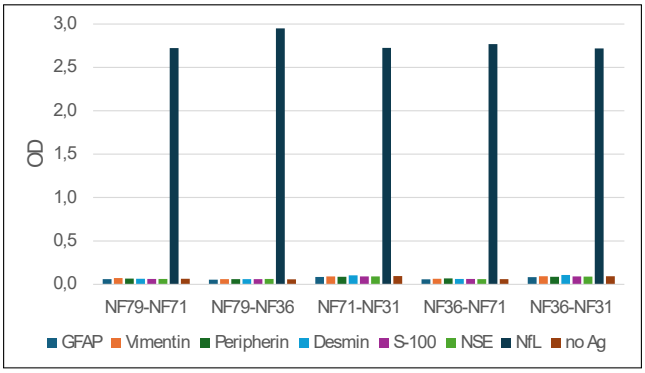


Figure 6.
Cross-reactivity of different prototype assays to various neuronal proteins. A three-step sandwich immunoassay format was used. Recombinant proteins were tested at a concentration of 1,000 ng/mL, with recombinant NfL included as a positive control.

Measuring NfL levels in clinical samples

Assays based on Hytest MAb pairs can be used to measure endogenous NfL in CSF samples. Figure 7 shows the dilution curve of endogenous NfL in CSF, measured using a prototype ELISA developed with the NF79–NF71 antibody pair. When used on highly sensitive immunoassay platforms, this antibody pair can also measure NfL levels in blood samples.

The prototype NF79–NF71 assay was also used to measure NfL in CSF samples from patients with various diagnoses. Depending on the diagnosis, the samples showed low, moderate, or high NfL levels, as presented in Figure 8 on page 4.

Correlation of anti-NfL assay prototypes with other assays

Prototype assays demonstrated good correlation with a commercial assay. NfL concentrations in 23 CSF samples from patients with various conditions were measured using the commercially available NF-light® ELISA (Uman Diagnostics) and three prototype assays:

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NF79–NF71, NF79–NF36, and NF36–NF71. Both the colorimetric prototype assays and the commercial assay are three-step sandwich ELISAs employing a biotinylated detection MAb. As shown in Figure 9, all Hytest prototype assays exhibited good linear correlations with the reference assay.

Anti-NfL antibodies for immunochemical staining

Anti-NfL MAb NF36 can also be used for ICC and IHC. Representative immunostaining results are shown in Figure 10.

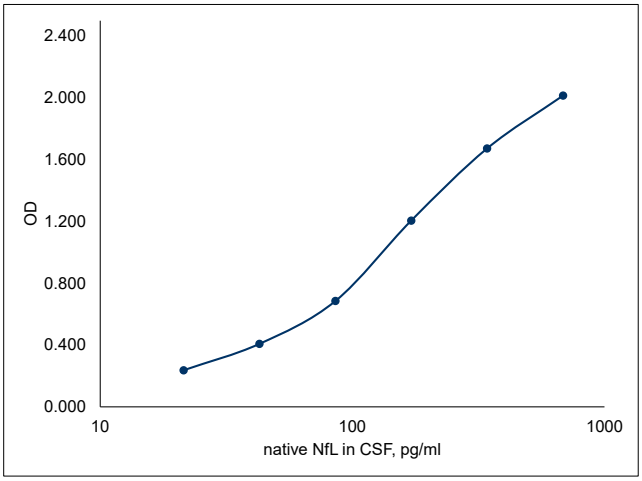
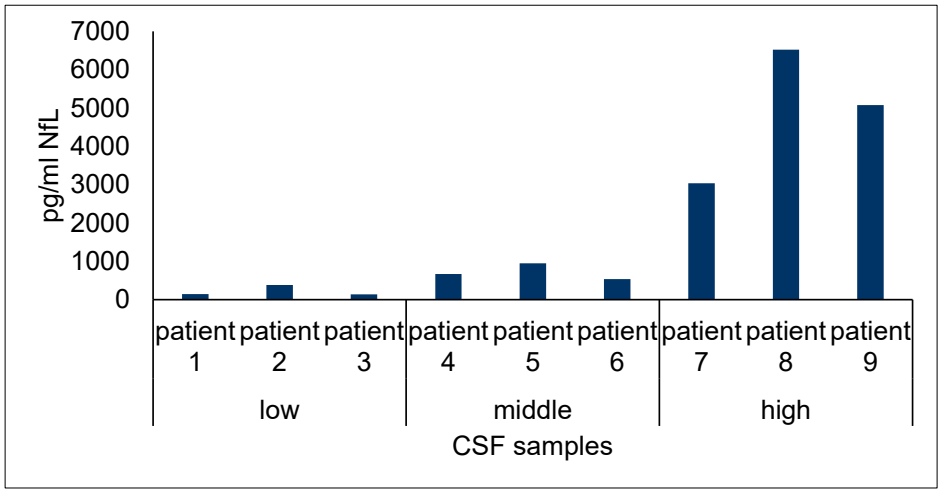


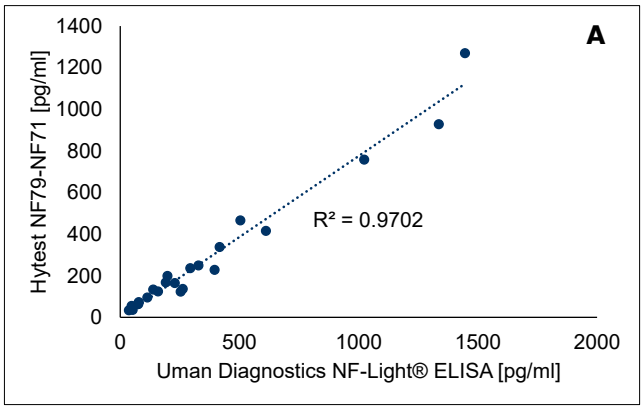
Figure 7.
Titration curve of endogenous NfL in a CSF sample measured using an in-house prototype sandwich immunoassay with NF79 as the capture antibody and NF71 as the detection antibody. The initial NfL concentration in the CSF sample was determined using the Uman Diagnostics NF-light® ELISA.

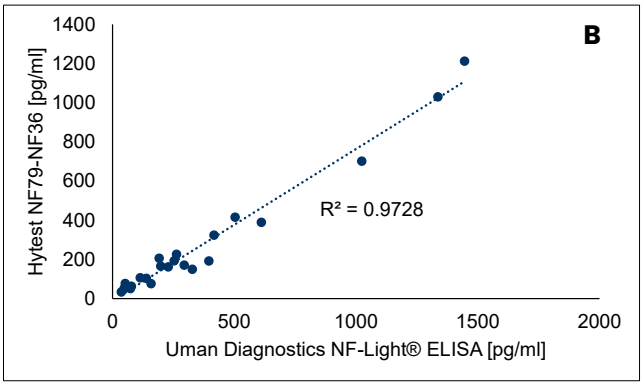


patient 1	migraine
patient 2	concussion
patient 3	pneumonia
patient 4	chronic cerebral ischemia
patient 5	unspecified stroke
patient 6	non-infectious encephalitis
patient 7	subarachnoid hemorrhage
patient 8	dyscirculatory encephalopathy
patient 9	Guillain-Barré syndrome

Figure 8.
NfL detection in CSF samples using a prototype three-step in-house sandwich immunoassay with NF79 as the capture antibody and NF71 as the detection antibody. The adjacent table lists each patient's diagnosis. CSF samples were diluted four- to five-fold before measurement.

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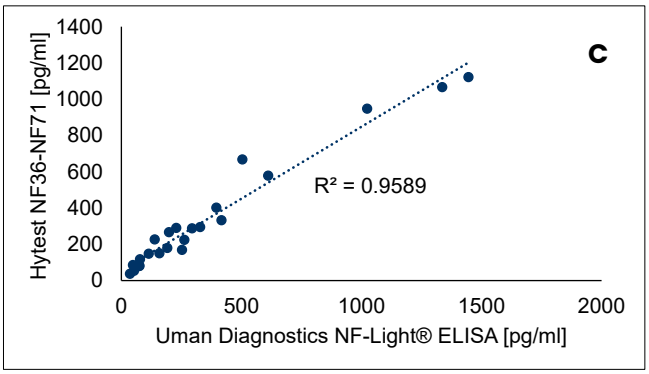


Figure 9.
Correlation of NfL measurement results in 23 CSF samples between the NF-light® ELISA (Uman Diagnostics) and three prototype ELISAs using Hytest antibodies: (A) NF79–NF71, (B) NF79–NF36, and (C) NF36–NF71.

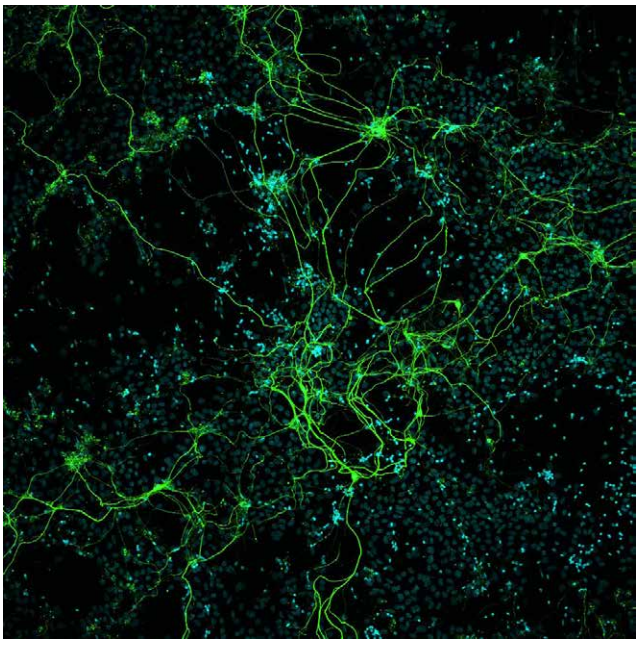


Figure 10.
Immunochemical staining of NfL in cultured murine neurons. Neurons were isolated from the hippocampus of a mouse embryo. NF36 was used as the primary antibody, and Alexa Fluor™ 488-conjugated anti-rabbit polyclonal antibodies were used for detection (green). Nuclei were counterstained with Hoechst 33342 (blue).

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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, monoclonal rat antibody

ANTIGEN

Product name	Cat. #	Purity	Source
Neurofilament light chain (NfL), human, recombinant	8NF5	≥95%	Recombinant

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HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • **HYTEST.FI**
HYTEST NORTH AMERICA INC.
800 MacArthur Blvd, STE 100, Mahwah, New Jersey 07430
Tel. 833-368-3489 • E-mail: hytestna@hytest.fi • **HYTESTNA.COM**