



10-7001

## Instructions for Use - NF-light® (Neurofilament light) ELISA for CSF samples

### 1. Intended use

NF-light® ELISA is an in vitro diagnostic device intended for quantitative determinations of human Neurofilament light (NF-L) protein in cerebrospinal fluid (CSF). Increased NF-L levels indicate nerve cell degradation, and the result is used to **aid the diagnosis** of neurological diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), dementias and Parkinson's disease (PD). The results from this assay must be used together with other clinical observations and patient anamnesis, since NF-L is an unspecific biomarker for axonal damage. The intended test population is people > 18 years who are suspected of suffering from a neurological disease. In addition, the NF-light® ELISA can be used for research using CSF samples containing NF-L from rat, bovine, mouse or macaque sources as the antibodies in the assay recognizes NF-L from these species as well.

The kit is intended for professional use, i.e. should be used by clinical laboratory personnel trained in ELISA technology and in vitro diagnostic procedures.

### 2. Notice to user

If a serious incident occurs in relation to this device, the incident should be reported to the manufacturer and to the appropriate local competent authority of the member state in which the user and/or patient is established. To report to the manufacturer, see contact details in the end of this instruction.

### 3. Summary and explanation

Neurofilaments are the main cytoskeletal constituents in neuronal cells. They are important for the maintenance of the axonal caliber and morphological integrity, which affects the velocity and fidelity of neuronal transmissions. Three different neurofilament chains exist, named according to their size. These are Neurofilament light, medium and heavy respectively. The Neurofilament light constitutes the backbone to which the heavier chains co-assemble, forming the neurofilament fiber [1]. Following injuries of nerve cells due to direct trauma or slow degenerative processes, the content of the cell is released into the surrounding compartment allowing quantitative determinations of the axonal proteins. Increased levels of Neurofilament light have been found in various degenerative diseases such as Amyotrophic lateral sclerosis, Alzheimer's disease, and Multiple sclerosis [2-4].

### 4. Method description

The UmanDiagnostics NF-light® ELISA assay is an enzymatic immunoassay designed for quantitative determinations of NF-L in human cerebrospinal fluid and cannot in its present form be used for analysis of blood samples. The test uses two highly specific non-competing monoclonal antibodies [5]. One specific monoclonal antibody is coated on a solid surface and binds NF-L. Detection is performed by use of another specific conjugated monoclonal antibody. Quantitative determinations are performed by enzymatic turn-over of a colorless substrate to a colored product, which corresponds to the amount of NF-L in the sample. The assay is not automated and uses traditional 96-well plates. Only standard laboratory equipment is required.

Standard curve range:	50 – 5000 pg/mL (including anchor points)
Standard curve quantification range:	125 pg/mL – 2500 pg/mL (samples diluted 1+1)
Detection limit:	33 pg/mL
Precision:	Intra-assay CV% < 5, Inter-assay CV% < 10
Incubation time:	2.5 hours
Sample size:	50 µL/replicate

### 5. Warnings, precautions and important notes


- In case of severe damage of the kit package please contact your supplier in written form no later than one week after receiving the kit. Do not use damaged components. Please keep the damaged components stored for complaint related issues.
- The NF-light® ELISA is for in vitro diagnostic use only and is not for internal use in humans or animals.
- There are no substances in the kit of animal or human origin that present a risk of infection.

- All human-sourced material should be considered potentially infectious and handled with caution. In case of spillage, immediately disinfect with 0.5% sodium hypochlorite or equivalent.
- The product should be used strictly in accordance with this instruction for use (IFU). Follow good laboratory practice and safety guidelines. Wear lab coats, disposable gloves and protective glasses when necessary.

## 6. Reagent handling

- The kit can be used at two separate analysis occasions. Reconstituted Standard and working solutions of Tracer and Conjugate are single use. After two analysis occasions, any unused reagents should be discarded.
- All assay reagents should be brought to room temperature prior to use.
- It is advised to run samples and standards in duplicate. If large deviations occur between replicates, please re-assay.
- Do not mix reagents of different lots. This can result in erroneous results.
- All incubation steps should be performed at room temperature (RT, +20-25 °C).

- **During the incubation steps, use an orbital ELISA table top shaker at 800 rpm. Agitation of the plate at 800 rpm is of UTMOST IMPORTANCE. Using a lower speed will result in falsely elevated results.**
- **Use the supplied 15 mL Sarstedt tube (62.554.502) when preparing the conjugate solution. Other tubes can have a negative impact on the stability of the solution causing the absorbance level to drop and sample read-outs to be unreliable.**

- Dispose all material which has been in contact with samples and reagents in accordance with country, state and local regulations.
-  **Warning** Avoid contact with Stop reagent. It may cause skin irritations and burns. Material Safety Data Sheet for this product is available on UmanDiagnostics website and can also be sent by email upon request.

## 7. Shelf-life and Storage of Reagents

The kits are shipped at ambient temperature. Upon arrival, they should be stored at +2-8 °C, kept away from heat or direct sunlight. Do not freeze the components. Once opened, the NF-light® strip plate should be used within 4 weeks. Make sure that an open strip plate is sealed to avoid humidity. The shelf-life for the kit is 18 months from date of production.

## 8. Sample Collection and Storage

All patient samples should be considered potentially contagious. After lumbar puncture the samples should be kept at -80 °C in polypropylene tubes. Repetitive freeze/thawing should be avoided.

The sample stability has been evaluated for 5 different clinical samples. The sample reactivity following different treatments was compared to the same sample stored at -80 °C.

		Mean % of -80 °C control	Mean % range
Freeze-thawing	≤ 4 cycles	98	96-101
Storage	5-8 °C ≤ 1 week	99.7	95-108
	24 h at RT (22 °C)	100	91-106
	-20 °C 1 month	95.8	89-109

## 9. Materials

### Kit components provided:

Short name	Full name	Description	Quantity
PLATE	Anti-NF-light strip plate	Pre-coated with mouse anti NF-L monoclonal antibody sealed in plastic pouch.	12 x 8 wells
STOP	Stop reagent	Diluted H <sub>2</sub> SO <sub>4</sub> (8% v/v).	1 x 6 mL
TMB	TMB substrate	Tetramethylbenzidine substrate.	1 x 12 mL
SAMDIL	Sample diluent	Aqueous buffered solution with detergent.	1 x 40 mL
CONDIL	Conjugate diluent	Aqueous buffered biotin free stabilizing solution.	1 x 12 mL
CONJ	Conjugate concentrate	Streptavidine Horseradish peroxidase conjugate in aqueous buffered biotin-free stabilizing solution. Dilute according to label.	1 x 260 µL
50xTRAC	Tracer concentrate (50x)	Biotin labelled anti NF-L monoclonal antibody in aqueous buffered biotin-free stabilizing solution.	1 x 260 µL
STAND	Bovine NF-L standard	Reconstitute according to the bottle label. (Contains BSE-, FMD-negative bovine material of German origin).	2 vials
10xWASH	Wash buffer concentrate (10x)	10x Aqueous buffered solution with detergent.	2 x 40 mL

### Additional material provided:

Plate cover 2 pcs

15 mL tube for conjugate dilution 2 pcs

### Not included essential equipment:

Microtiter plate reader 450 nm (reference wavelength 620-650 nm)

Micropipettes 10-1000 µL

Vortex mixer

Orbital ELISA table top shaker (800 rpm)

Deionized water

Wash bottle, automated or semi-automated microtiter plate wash system

Pipette tips and timer

Polystyrene or polypropylene tubes for standard and sample dilution

## 10. Assay procedure

### Preparations:

**Preparation of wash buffer 1x:** Dilute the total content of one 10x Wash buffer concentrate (10xWASH) bottle with deionized water to a final volume of 400 mL. Diluted, unused wash buffer can be stored at room temperature and should be used within two months. The 10x Wash buffer concentrate can appear opalescent due to high salt concentration (no effect on assay performance).

### Preparation of standard dilution series:

Reconstitution and preparation of the standard dilution series should be performed directly before use. Standard material should not be stored and re-used. Standard curves should be included on every plate analyzed.

The highest standard point (5000 pg/mL) is obtained by reconstituting one vial of lyophilized Standard (STAND) with the volume of sample diluent (SAMDIL) indicated on the vial label. Vortex briefly and keep in room temperature.

Label 7 micro-tubes, one each for the additional standard points (that is 2500 pg/mL, 1250 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 50 pg/mL and 0 pg/mL) and dilute the reconstituted standard according to the table below using sample diluent (SAMDIL).

Make a serial dilution as described below.

Level no.	Concentration pg/mL	Sample Diluent (SAMDIL)	Standard from tube no.
1 (vial)	5000	Reconstitute with Sample diluent (SAMDIL) according the Standard vial label	
2	2500	300 µL	300 µL (1, vial)
3	1250	300 µL	300 µL (2)
4	500	360 µL	240 µL (3)
5	250	300 µL	300 µL (4)
6	125	300 µL	300 µL (5)
7	50	360 µL	240 µL (6)
8	0	300 µL	0 µL

#### Assay overview:

	Washing 3 x 300 µL		
Samples, Standards and Controls	CSF-samples / Internal Control sample (1+1 dilution)	Standards (no. 1-7)	Blank (SAMDIL/no. 8)
	100 µL	100 µL	100 µL
	Incubation 1 hour, 800 rpm		
	Washing 3 x 300 µL		
Tracer Ab 1x	100 µL		
	Incubation 45 minutes, 800 rpm		
	Washing 3 x 300 µL		
Conjugate 1x	100 µL		
	Incubation 30 minutes, 800 rpm		
	Washing 3 x 300 µL		
TMB	100 µL		
	Incubation 15 minutes, 800 rpm		
Stop Solution	50 µL		
	Read the plate at 450 nm (reference wavelength 620-650 nm) directly after adding the Stop Solution		

#### Detailed assay protocol:

1. Dilute the CSF samples with equal amount (1+1) of Sample diluent (SAMDIL) to a total minimum volume of 210 µL. The standards, reconstituted and diluted according to the standard dilution table, are ready to use (i.e., no further dilution should be made).
2. Wash the wells to be used with Wash buffer 1x (3x300 µL). Washing could be performed either by an automated washer or by manual pipetting.
3. Add 100 µL of each Standard (8 levels including blank) and sample in duplicate. Incubate 1 hour at RT with agitation (800 rpm).
4. Wash the wells with Wash buffer 1x (3x300 µL), see point 2.

5. Directly before use, dilute the concentrated Tracer 50x (50x TRAC) to 1x with Sample diluent (SAMDIL). Mix thoroughly by inverting the tube or by vortexing. Add 100 µL of freshly diluted Tracer antibody to each well. Incubate 45 minutes at RT with agitation (800 rpm).
6. Wash the wells with Wash buffer 1x (3x300 µL), see point 2.
7. Directly before use, dilute the concentrated Conjugate (CONJ) in the supplied Sarstedts 15 mL tube according to the vial label with Conjugate diluent (CONDIL) to 1x. Mix thoroughly by inverting the tube or by vortexing. Add 100 µL of newly diluted Conjugate to each well. Incubate 30 minutes at RT with agitation (800 rpm).

**Important information: Use only the supplied 15 mL tube when preparing the conjugate solution.**

8. Wash the wells with Wash buffer 1x (3x300 µL), see point 2.
9. Add 100 µL of TMB to each well. Incubate 15 minutes at RT with agitation (800 rpm).
10. Add 50 µL of Stop reagent (STOP) to each well and read the absorbance at 450 nm (reference wavelength 620-650 nm).



**The stop reagent contains diluted sulfuric acid and is corrosive.**

### 11. Calculation of results

The results can be calculated automatically by using an immunoassay software package. A  $1/y^2$  – weighted 4-parameter algorithm provides the best curve fit (see a typical standard curve below). If no such immunoassay software is available, the concentration of NF-L is calculated from plotting average OD at ( $\lambda$  450 minus  $\lambda$  reference) against the known standard concentrations.

**The concentration from the standard curve should be multiplied by 2 to obtain the concentration in the sample (due to dilution 1+1 before analysis).**

### 12. Dilution

Samples displaying concentrations above 5000 pg/mL need to be further diluted and re-assayed. Based on the initial result, a dilution factor should be chosen to achieve a concentration in the range 125 – 2500 pg/mL. The outermost standard points (50 and 5000 pg/mL) are anchor points serving only to generate a more precise curve fit. Quantification between the two second outermost standard points (125 and 2500 pg/mL) and their respective anchor point should not be done. The most accurate quantification is obtained when measurements are within the standard curve range 125 to 2500 pg/mL. The result obtained from the standard curve is multiplied with the dilution factor used.

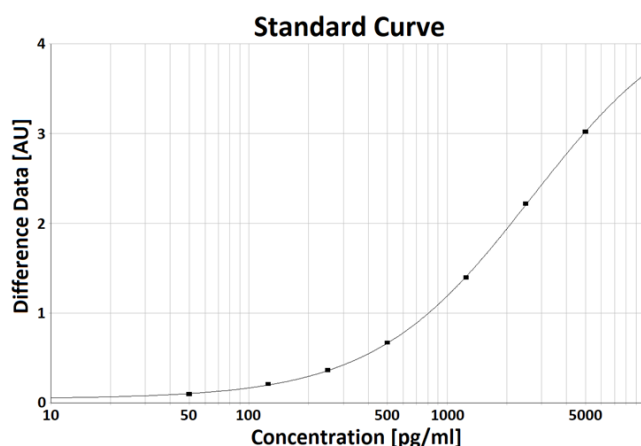
### 13. Quality Control

In order to verify the kit performance, the following criteria should be fulfilled for each analysis occasion;

- The curve should have an appearance as shown in the figure below.
- The maximum absorbance for 5000 pg/mL should be > 2.0 AU.
- The background should be <0.1 AU.

Internal control samples from healthy controls and/or samples containing elevated levels from patients should be established if the kit is used in clinical routine analysis. It is recommended that at least one control sample in the concentration range 1000-3000 pg/mL is established. Control samples can be prepared by pooling samples of cerebrospinal fluid and analyzing the pool repeatedly to establish concentration levels and acceptance criteria. The pool should be aliquoted and stored at -80 °C.

Below, a typical standard curve at time of release is shown, and approximate absorbance values given.



Standard level (pg/mL)	% of maximum signal for 5000 pg/mL.
5000 (anchor point)	100
2500	74
1250	47
500	22
250	12
125	6.5
50 (anchor point)	3.7

## 14. Measuring range

The standard curve covers the interval 50 - 5000 pg/mL NF-L. The 5000 pg/mL and 50 pg/mL standards serve as anchor points and quantification should be performed within the range 125 - 2500 pg/mL of the standard curve, taking into account the dilution factor of the sample, this corresponds to 250 - 5000 pg/mL of NFL in the original sample. Extrapolation beyond the curve is not allowed with the implication that samples outside of the curve must be further diluted and re-measured.

## 15. Limitations of Use

For clinical samples the following criteria should be taken into consideration;

- NF-L levels are markedly elevated in atypical PD compared to PD [6].
- Different types of dementia are associated with different levels of NF-L [7].
- In case of any diagnostic procedure, the results from this assay must be interpreted together with other clinical findings.

Potential interference from heterophilic antibodies might cause erroneous results. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce human anti-animal antibodies, e.g. HAMA, that interfere with this immunoassay. Another potential source of interference is if patients have received biotin therapy. Carefully evaluate results if the samples are suspected of having these types of interferences.

## 16. Clinical evaluation

Levels of NF-L in CSF have been analyzed for 35 different neurological and psychiatric conditions using UmanDiagnostics NF-light® ELISA kit [8]. The meta study was based on 47 data sets and include data from 10 059 individuals.

The results showed that NF-L levels were increased compared to healthy controls for most of the conditions, the exceptions were;

- Parkinson's disease ( $p > 0.95$ )
- Parkinson's disease dementia ( $p > 0.95$ )
- Dementia with Lewy bodies LB ( $p = 0.09$ )
- Primary progressive MS ( $p = 0.33$ )
- Idiopathic normal pressure hydrocephalus ( $p > 0.95$ )
- Mild cognitive impairment ( $p = 0.10$ )
- Chronic inflammatory polyradiculopathy/Guillain Barré syndrome

The highest levels of NF-L were observed for;

- HIV
- Frontotemporal dementia/ALS
- ALS
- Huntington disease
- Frontotemporal dementia

The concentration levels overlapped in most clinically similar diagnosis except for;

- Frontotemporal dementia and HIV with cognitive impairment which differed from other dementias
- Parkinson's disease, which segregated from atypical parkinsonian syndromes.

The levels of healthy controls were dependent on age and gender.

In healthy controls, NF-L levels in CSF are known to increase with age due to axonal degradation. Experience from clinical routine analysis since the early development of the product (2008) has resulted in the following cut-off levels;

Age		Reference value
Adults	< 30 years	< 380 pg/mL
	30 – < 40 years	< 560 pg/mL
	40 – < 60 years	< 890 pg/mL
	≥ 60 years	< 1850 pg/mL

The results are only valid if the test has been performed according to the instructions for use and must be correlated to other clinical observations and diagnostic tests. The user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws.

## 17. Performance data

### Traceability of standard

The test is standardized using internal quality control samples of cerebrospinal fluid from patients (pooled samples). No reference method or standard reference material is commercially available. Below, typical batch-to-batch variation for the absorbance and QC-samples are shown.

Kit lot	Abs 5000 pg/mL (AU)	Conc. QC-sample 1 (pg/mL)	Conc. QC-sample 2 (pg/mL)	Conc. QC-sample 3 (pg/mL)
70668/70678	3.23	4079		2245
70688/70698	3.06	4246		2262
70716/70726	3.17	4151		2335
70736/70746	3.27	4571		2384
70784/70794	2.95	3893	2211	
70804/70814	3.12	3944	2275	
70836/70845	3.02	4440	2315	
<b>Mean:</b>	<b>3.12</b>	<b>4189</b>	<b>2267</b>	<b>2307</b>
<b>SD:</b>	<b>0.12</b>	<b>245</b>	<b>45</b>	<b>65</b>
<b>CV:</b>	<b>3.8 %</b>	<b>5.8 %</b>	<b>2.0 %</b>	<b>2.8 %</b>

### Specificity

CSF samples have been spiked with 50 000 pg/mL neurofilament medium (NF-M) and neurofilament heavy (NF-H). Recovery of NF-L in NF-M and NF-H spiked samples varied between 95.1 – 103 %.

### Analytical Sensitivity

Limit of Detection (LoD) 33 pg/mL, Limit of Quantitation (LoQ) 81 pg/mL

### Precision

Intra-precision of NF-light® ELISA: < 5 % (700 – 5000 pg/mL)

Inter-precision of NF-light® ELISA: < 10 % (700 – 5000 pg/mL)

### Dilution linearity

There is dilution linearity in the concentration interval 53 – 21 000 pg/mL.

### Parallelism

Dilution of CSF samples follow the same trend as dilution of spiked samples. Dilution does not affect concentration determination of endogenous NFL in the investigated concentration interval 171 – 6900 pg/mL.

### Recovery

The recovery in the investigated NFL concentration interval 1700 – 6800 pg/mL is between 88-108 %.

### Accuracy

It has not been possible to compare the results for this assay with any other method as no CE marked kit or standard reference material for NF-L are available.













## 18. Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by UmanDiagnostics AB may affect the results, in which event UmanDiagnostics AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. UmanDiagnostics AB and its authorized distributors, in such event, shall not be liable for any damages, whether direct, indirect or consequential.

## 19. Bibliography

1. Yuan, A., et al., *Neurofilaments and Neurofilament Proteins in Health and Disease*. Cold Spring Harb Perspect Biol, 2017. **9**(4).
2. Feneberg, E., et al., *Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis*. Neurology, 2018. **90**(1): p. e22-e30.
3. Andersson, E., et al., *Blood and cerebrospinal fluid neurofilament light differentially detect neurodegeneration in early Alzheimer's disease*. Neurobiol Aging, 2020. **95**: p. 143-153.
4. Gunnarsson, M., et al., *Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab*. Ann Neurol, 2011. **69**(1): p. 83-9.
5. Norgren, N., et al., *Monoclonal antibodies selective for low molecular weight neurofilaments*. Hybrid Hybridomics, 2002. **21**(1): p. 53-9.
6. Hall, S., et al., *Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders*. Arch Neurol, 2012. **69**(11): p. 1445-52.
7. Khalil, M., et al., *Neurofilaments as biomarkers in neurological disorders*. Nat Rev Neurol, 2018. **14**(10): p. 577-589.
8. Bridel, C., et al., *Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis*. JAMA Neurol, 2019.

## 20. Symbols used

	Cat.-No.:
	Use by:
	Lot-No.:
	No. of Tests:
	In Vitro Diagnostic Medical Device
	Read instructions before use
	Keep away from heat or direct sun light.
	Store at:
	Manufacturer:
	Country of manufacture:
	Caution!
	Warning



**UmanDiagnostics AB**  
**Tvistevägen 48C**  
**907 36 Umeå, Sweden**

**Phone: +46(0)90 777 880**  
**info@umandiagnostics.com**  
**www.umandiagnostics.com**

Instructions for use in other languages are available for direct download at company website.