

## Instructions for Use



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# NF-light® (Neurofilament light) ELISA

**Enzyme immunoassay for the quantitative determination of Neurofilament light (NF-L) in cerebrospinal fluid.**

**REF 30112458**

**Σ 12 x 8**

**For illustrative purposes only.**

**To perform the assay the instructions for use provided with the kit have to be used**

**For research use only.**

**Not for use in diagnostic procedures.**

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Flughafenstrasse 52a  
22335 Hamburg, Germany

**Always there for you**



## Research Use Only

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

#### 1. Limited use statement

BY USE OF THIS PRODUCT, RESEARCHERS AGREE TO BE BOUND BY THE TERMS OF THIS LIMITED USE STATEMENT. Researchers may use this product for research use only, not for diagnostic procedures, no commercial use is allowed. Commercial Use means any and all uses of this product and derivatives by a party for monetary or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research.

#### 2. Intended use

NF-light™ ELISA is an enzyme immunoassay intended for quantitative determinations of human Neurofilament light (NF-L) protein in cerebrospinal fluid (CSF). In addition, the NF-light™ ELISA can be used for research using samples containing NF-L from rat, bovine or macaque sources as the antibodies in the assay recognizes NF-L from these species as well. The kit is intended for professional use.

#### 3. Notice to user

If a serious incident occurs in relation to this device, the incident should be reported to the manufacturer, see contact details in the end of this instruction.

#### 4. 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.

#### 5. 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 [2]. 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

## 6. 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 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.

## 7. Reagent handling

- The kit can be used on 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.

## 8. 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.

## 9. 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

## 10. 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

## 11. 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 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.**

## 9. 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).**

## 10. 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 – 2500 pg/mL. The result obtained from the standard curve is multiplied with the dilution factor used.

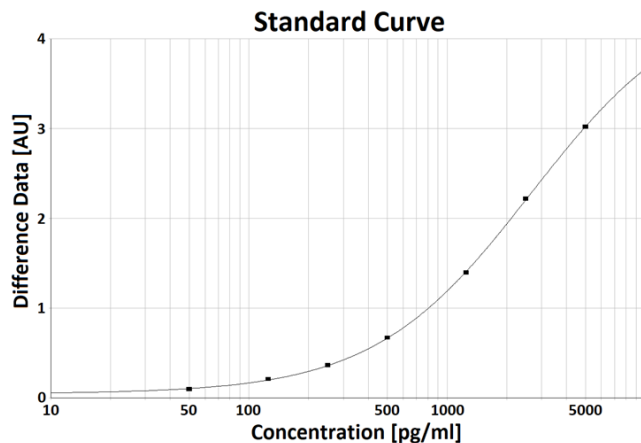
## 11. Quality Control

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.

It is recommended that at least one control sample in the concentration range 1000-3000 pg/mL should be 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 relative approximate absorbance values are 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

## 12. 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.

## 13. Limitations of Use

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.

## 14. 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 Quantification (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**

There is no standard reference material available for NF-light.












**15. 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.

**16. Bibliography**

1. Yuan, A., et al., *Neurofilaments and Neurofilament Proteins in Health and Disease*. Cold Spring Harb Perspect Biol, 2017. **9**(4).
2. Norgren, N., et al., *Monoclonal antibodies selective for low molecular weight neurofilaments*. Hybrid Hybridomics, 2002. **21**(1): p. 53-9.

**17. Symbols used**


















	Catalogue number
	Use by date
	Batch code
	Contains sufficient for <n> tests
	Consult instructions for use
	Keep away from sunlight
	Temperature limit
	Manufacturer
	Country of manufacture
	Caution
	Warning



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# Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.-Cat.: / N.º Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλοποιημένο
	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
	Unique Device Identification / Eindeutige Geräteerkennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazena a: / Conservare a: / Armazena em: / Αποθήκευση στους:
	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazena a: 2 - 8°C / Conservare a: 2-8°C / Armazena em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symboles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

**LIMITATION OF LIABILITY:** IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.

For further country-specific classifications, please refer to the corresponding safety data sheet.



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