UmanDiagnostics A Quanterix Company

Research Use Only

REF 10-7002

# 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 $H_2SO_4$ (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 (λ 450 minus λ 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.

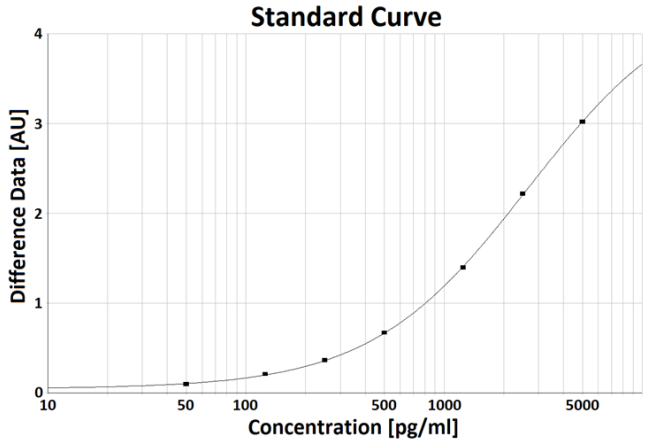
## 11. Quality Control

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 | |
| **Mean:** | **3.12** | **4189** | **2267** | **2307** |
| **SD:** | **0.12** | **245** | **45** | **65** |
| **CV:** | **3.8 %** | **5.8 %** | **2.0 %** | **2.8 %** |

**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

| | |
|---|---|
| REF | Catalogue number |
| | Use by date |
| LOT | 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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