UmanDiagnostics
Rapid detection of brain disease

REF 10-7002

Instructions for Use - NF-light® (Neurofilament light) ELISA

1. Limited use statement

BY USE OF THIS PRODUCT, RESEARCHERS AGREES 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). The kit is intended for professional use.
In addition, the NF-light® ELISA can be used for research using samples containing NF-L from rat, bovine and macaque sources as the antibodies in the assay recognizes NF-L from these species as well.

3. Method description

The UmanDiagnostics NF-light® (Neurofilament light) ELISA is an enzymatic immunoassay designed for quantitative determinations of NF-L in human cerebrospinal fluid. The test uses two highly specific non-competing monoclonal antibodies (Norgren et al., 2002). 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.

Measuring range:	100 pg/mL – 10 000 pg/mL
Detection limit:	33 pg/mL
Precision:	Intra-assay CV% < 5, Inter-assay CV% < 10
Incubation time:	2.5 hours
Sample size:	50 µL/replicate

4. Warnings and precautions

The NF-light® ELISA is for laboratory use only and is not for internal use in humans or animals.

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 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.

CAUTION: Avoid contact with Stop reagent. It may cause skin irritations and burns. Material Safety Data Sheet for this product is available upon request directly from UmanDiagnostics.

5. Handling precautions

- The kit has been designed to be able to be used at two separate analysis occasions.
- 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.
- During the incubation steps, use an orbital ELISA table top shaker at 800 rpm. **Agitation of the plate at 800 rpm is of HIGH IMPORTANCE.**
- All incubation steps should be performed at room temperature (RT, +20-25 °C)
- 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.

Shelf-life and Storage of Reagents
Store the kit at +(2-8) °C and keep away from heat or direct sunlight. Do not freeze the components.
Reconstituted standard should be used immediately and cannot be re-used.
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 is 18 months from date of production.

6. Sample Collection and Storage

The NF-light® ELISA test is developed for analysis of cerebrospinal fluid samples and cannot in it's present form be used for analysis of blood samples. 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

7. 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
Absorbent paper, pipette tips and timer
Polystyrene or polypropylene tubes for standard and sample dilution

8. Assay procedure

Preparations:

- All assay reagents should be brought to room temperature prior to use.
- **Preparation of wash buffer;** 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 (calibrators):
Reconstitution and preparation of standard dilution series should be performed directly before use.
Label 6 micro-tubes, one for each standard point (that is 5 000 pg/mL, 2 500 pg/mL, 1 000 pg/mL, 500 pg/mL, 100 pg/mL and 0 pg/mL). The highest standard point (10 000 pg/mL) is obtained by reconstituting one vial of lyophilized Standard (STAND) with the volume of sample diluent (SAMDIL) indicated on the bottle label. Vortex briefly and keep in room temperature.

Make a serial dilution as described below.

Tube no.	Concentration (pg/mL)	Sample Diluent (SAMDIL)	Calibrator from tube no.
Vial	10 000	Reconstitute with Sample diluent (SAMDIL) according the standard vial label	
1	5 000	300 µL	300 µL (vial)
2	2 500	300 µL	300 µL (1)
3	1 000	360 µL	240 µL (2)
4	500	300 µL	300 µL (3)
5	100	240 µL	60 µL (4)
6	0	300 µL	0 µL

Running the assay:

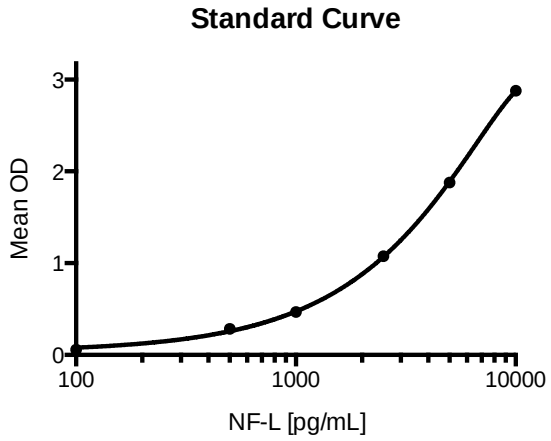
1. Dilute the CSF samples with equal amount (1+1) of Sample diluent (SAMDIL) to a total minimum volume of 210 µL. The standards reconstituted and diluted according to the standard dilution table are ready to use (i.e. no further dilution should be made).
2. Wash the wells to be used with Wash buffer (3x300 µL). The Wash buffer added could be either aspirated or removed by knocking the plate against absorbing material immediately before next washing cycle.
3. Add 100 µL of each Standard and sample in duplicate. Incubate 1 hour at RT with agitation (800 rpm).
4. Wash the wells with Wash buffer (3x300 µL), see point 2.
5. Directly before use, dilute the concentrated Tracer (50x TRAC) 1:50 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 (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). 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 (3x300 µL), see point 2.
9. Add 100 µL of TMB substrate (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. The 4-parameter Marquardt transformation 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 (λ450 minus λ620) against the known standard concentrations.

⚠ **The concentrations of NF-L in the samples are obtained directly from the calibration curve, the dilution of the sample (1+1) has already been compensated for and no multiplication should be done.**

10. Quality Control



4-parameter Marquardt transformation

In order to assure a high performance of the kit, the following criteria should be fulfilled;

- The curve should have an appearance as shown in the figure above.
- The maximum absorbance for 10 000 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.

11. Measuring range

The standard curve covers the interval 100 -10 000 pg/mL NF-L. Extrapolation beyond the curve is not allowed with the implication that samples outside the curve have to be further diluted and re-measured.

12. Dilution

Samples displaying concentrations above the highest standard point needs to be further diluted and re-assayed. When assaying a sample with expected levels above the standard curve, the sample should first be diluted with an equal amount of sample diluent according to point 1 in assay instructions. This should be followed by a second appropriate dilution to allow the concentration to reach levels covered by the standard curve. The concentration read from the curve needs to be multiplied by the dilution factor applied in the second step. Best results are usually obtained when omitting measurement in the far low and high standard range.

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. Carefully evaluate the results of patients suspected of having these antibodies. 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. 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.

15. Bibliography

Lee MK, Xu Z, Wong PC, Cleveland DW. Neurofilaments are obligate heteropolymers in vivo. J Cell Biol. 1993 Sep;122(6):1337-50.

Norgren N, Karlsson JE, Rosengren L, Stigbrand T. Monoclonal antibodies selective for low molecular weight neurofilaments. Hybrid Hybridomics. 2002 Feb; 21(1): 53-9

16. Symbols used

REF	Cat.-No.:
	Use by:
LOT	Lot-No.: /
	No. of Tests:
	Read instructions before use.
	Keep away from heat or direct sunlight.
	Store at: /
	Manufacturer: /
	Caution! /

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