

NF-light[®] **(Neurofilament light)** **RUO ELISA**

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

REF **30112458**

 **12 x 8**

For illustrative purposes only.

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

Distributed by:

NF-light® (Neurofilament light) ELISA

Enzyme immunoassay for quantitative determination of human Neurofilament light (NF-L) protein in cerebrospinal fluid. The antibodies cross-react with Neurofilament light from rat, bovine and macaque sources and can be used for research on the above species as well.

REF 10-7002

 12x8

   2-8 °C

U.S.: For research use only

Not for use in diagnostic procedures



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Intended use

Enzyme Immunoassay for quantitative determinations of human Neurofilament light (NF-L) protein in cerebrospinal fluid. The antibodies cross-react with Neurofilament light from rat, bovine and macaque sources and can be used for research on the above species as well.

Introduction

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

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 fibre (Lee et al., 1993).

The assay is a 2-site solid phase sandwich ELISA. One specific monoclonal antibody is coated on a solid surface and binds Neurofilament light. 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 Neurofilament light in the sample.

Measuring range:	100 pg/mL – 10000 pg/mL
Incubation time:	2.5 hours
Sample size:	50 µL/replicate
Storage temperature:	2-8 °C Keep away from heat or direct sunlight
Shelf life:	18 months from date of production

Once opened the NF-light® strip plate should be used within 4 weeks. Make sure that opened strip plate is sealed to avoid humidity.

Warnings and precautions

1. For research use only. For professional use only.
2. Before starting the assay, read the instructions for use completely and carefully. Use the valid version of the package insert provided with the kit. Make sure that everything is understood.
3. 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.
4. Do not mix reagents of different lots. Do not use expired reagents.
5. Please use the supplied 15 ml Sarstedt tube (62.554.502) when preparing the conjugate solution.
6. It is advised to run samples, controls and standards in duplicate. If large deviation occurs between samples, please re-assay.
7. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable gloves and protective glasses when necessary.
8. 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.

9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
10. Use an orbital ELISA table top shaker at 800 rpm.

Kit contents:

Content	Kit component	Quantity
	Plate cover	2 pcs
PLATE	Anti NF-light strip plate	12 x 8 wells
STOP	Stop reagent	1 x 6 mL
TMB	TMB substrate	1 x 12 mL
SAMDIL	Sample diluent	1 x 40 mL
CONDIL	Conjugate diluent	1 x 12 mL
CONJ	Conjugate concentrate	1 x 260 µL
50xTRAC	Tracer concentrate (50x)	1 x 260 µL
STAND	Bovine NF-L standard	2 vials
10xWASH	Wash buffer concentrate (10x)	2 x 40 mL

Chromogen and stop reagents

Enzyme: Horseradish peroxidase (HRP)

Substrate: Tetramethylbenzidine, TMB

Stop reagent: H₂SO₄ 8% v/v (**Warning corrosive**)

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)

Deionised 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

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

Properties of the test

Types of samples

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

Temperature dependence

The assay has been developed for room temperature (20 – 25°C).

Measuring range

The standard curve covers the interval 100 -10000 pg/mL NF-L (see curve on page 5). Extrapolation beyond the curve is not allowed with the implication that samples outside the curve have to be further diluted and re-measured.

Reagent preparation

Wash buffer

Dilute the total content of one 10x wash buffer concentrate bottle (10xWASH) with deionised 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 concentrate can appear opalescent due to high salt concentration (no effect on assay performance).

Standards

Reconstitution and preparation of standard dilution series should be performed directly before use. The highest standard point (10000 pg/mL) is obtained by reconstituting one vial of lyophilized standard (STAND) with the volume of sample diluent indicated on the bottle label. Vortex briefly and keep in room temperature. Label 6 micro-tubes, one for each standard point (that is 5000 pg/mL, 2500 pg/mL, 1000 pg/mL, 500 pg/mL, 100 pg/mL and 0 pg/mL). Dilute the standard according to the dilution table below:

Standard dilution:

Tube no.	Sample Diluent (SAMDIL)	Standard from tube no.	Concentration pg/mL
Vial	Reconstitute with sample diluent (SAMDIL) according the standard vial label		10000
1	300 µL	300 µL (vial)	5000
2	300 µL	300 µL (1)	2500
3	360 µL	240 µL (2)	1000
4	300 µL	300 µL (3)	500
5	240 µL	60 µL (4)	100
6	300 µL	0 µL	0

Reconstituted standard should be used immediately and cannot be re-used.

Tracer

Directly before use, dilute the concentrated tracer (50x TRAC) (biotin anti NF-L mAb) 1:50 with sample diluent (SAMDIL). Mix thoroughly by inverting the tube or by vortexing.

Conjugate

Directly before use, dilute the concentrated conjugate (CONJ) (Streptavidin-HRP conjugate concentrate) 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.

Assay instructions

All assay reagents should be brought to room temperature prior to use.

After each washing cycle the plate should be tapped dry against absorbent paper.

Agitation of the plate at 800 rpm is of HIGH IMPORTANCE.

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 room temperature (20-25°C) with agitation (800 rpm).
4. Wash the wells with wash buffer (3x300 µL), see point 2.
5. Add 100 µL of freshly diluted tracer antibody to each well. Incubate 45 minutes at room temperature (20-25°C) with agitation (800 rpm).
6. Wash the wells with wash buffer (3x300 µL), see point 2.
7. Add 100 µL of newly diluted conjugate to each well. Incubate 30 minutes at room temperature (20-25°C) with agitation (800 rpm).
8. Wash the wells with wash buffer (3x300 µL), see point 2.
9. Add 100 µL of TMB to each well. Incubate 15 minutes at room temperature (20-25°C) 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.

Changes in performance or assay characteristics

Any putative changes in performance and assay characteristics will be described in the instructions for use specific for each kit.

Samples

The CSF should not be contaminated with blood. 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.

Sample stability

The sample stability was evaluated in 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

Results

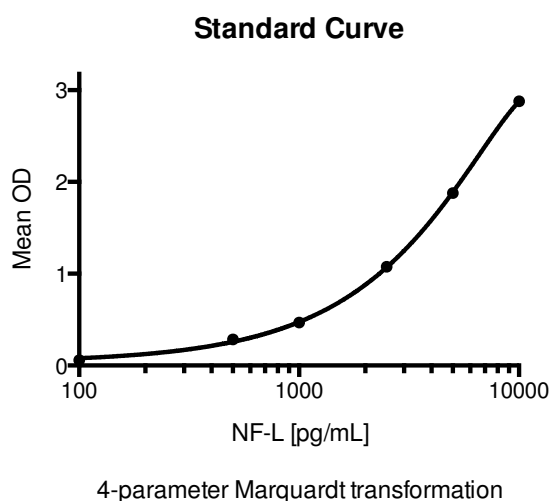
The results can be calculated automatically by using an immunoassay software package. The 4-parameter Marquardt transformation provides the best curve fit (see a typical standard curve below). If no such immunoassay software is available, the concentration of NF-L is calculated from plotting average OD at (λ450 minus λ620) against the known standard concentrations.

All test levels are obtained directly from the immunoassay software package, or from the manually plotted curve. 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.



The values obtained from the standard curve correspond directly to the NF-L level.



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.

References:














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

Symbols / Symbole / Symboles / Simboli / Simbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Use by: / Verwendbar bis: / Utilisé à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / 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. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
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	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p style="text-align: center;">Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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.

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