NEFL (Human) ELISA Kit

Catalog Number KA1478

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

NEFL (Human) ELISA Kit is intended for detection of the light-chain neurofilament proteins (NF-L) in cerebrospinal fluid.

Background

NF-L can be used as a marker for neuronal degeneration in animal brains as well as in human cerebral diseases. For instance, significantly increased amount of NF-L was found in cerebrospinal fluid (CSF) of vascular dementia, frontotemporal dementia, amyotrophic lateral sclerosis, olivopontocerebellar atrophy, normal pressure hydrocephalus, cerebral infarction, multiple sclerosis, Parkinson and Alzheimer disease, dementia with Lewy bodies, as well as in Lyme neuroborreliosis patients.

Principle of the Assay

NEFL (Human) ELISA Kit is a solid-phase immunoanalytical test based on the use of specific polyclonal and monoclonal NF-L antibodies that bind light-chain neurofilament proteins (NF-L). NF-L present in the sample of cerebrospinal fluid is bound during the first incubation by the NF-L antibodies, which are immobilized to the microtitrate wells. After the first incubation, the unbound NF-L is decanted and the wells are thoroughly washed. The second incubation is with monoclonal anti-NF-L antibodies. Subsequently, the biotin-bound antibody is used for amplification of reaction and for visualization of system are used the streptavidin-bound alkaline phosphatase and 4-nitrophenyl phosphate buffer as a substrate for alkaline phosphatase.

Flow Chart

Step 1.	Prepare reagents and samples
	↓
Step 2.	Wash 3 times (250 µL/well), aspirate
	↓
	Add 100 µL/ well of Standards (St A – ST F) and samples
	↓
	Incubate 2 hours at 37°C
	↓
Step 3.	Wash 5 times (250 µL/well), aspirate
	↓
	Add 100 µL/ well of mixture of IgG 1 with IgG 2
	↓
	Incubate 60 min at 37°C
	↓
Step 4.	Wash 5 times (250 µL/well), aspirate
	↓
	Add 100 µL/ well of IgG-biot
	↓
	Incubate 60 min at 37°C
	↓
Step 5.	Wash 5 times (250 µL/well), aspirate
	↓
	Add 100 µL/ well of Str-AP
	↓
	Incubate 30 min at 37°C
	↓
Step 6.	Wash 5 times (250 µL/well), aspirate
	↓
	Add 100 µL/ well of 4-nitrophenyl phosphate buffer
	↓
	Incubate 60 min at room temperature
	↓
Step 7.	Read optical density for 450 nm till 5 min

General Information

Materials Supplied

List of component

Component	Amount
16-well strips (colourless) coated with the specific polyclonal antibody within a plastic frame	1 microplates
NF-L antigen – Standard (NF-L), lyophilized powder	1 vial
mouse anti-NF-L IgG antibody 1 (IgG 1), 100x concentrated	0.2 mL
mouse anti-NF-L IgG antibody 2 (IgG 2), 100x concentrated	0.2 mL
rabbit anti-mouse IgG antibody with biotin (anti-IgG-biot), 100x concentrated	0.2 mL
alkaline phosphatase – streptavidin (Str-AP), 100x concentrated	0.2 mL
4- nitrophenyl phosphate buffer, r.t.u.*	15 mL
Wash buffer concentrate, 10x concentrated	125 mL
Dilution buffer, r.t.u.*	125 mL
Sealable pouch for unused strips	1 piece

*(read to use)

Storage Instruction

- ✓ Store the kit reagents at 2 to 10°C, in a dry place and protected from the light. Avoid freezing. Expiration date is indicated at the ELISA kit label and at all reagent labels.
- ✓ Store unused strips in the sealable pouch and keep the desiccant inside. Transport in thermo bags until 72 hours. Any damages of packaging of kit reagents advise to the producer without delay.
- ✓ Do not store diluted samples of cerebrospinal fluid, Standards (ST A – ST F), IgG 1, IgG 2, IgG-biot, and Str-AP. Always prepare fresh. The diluted Wash buffer store 1 week at 2 to 10°C.

Materials Required but Not Supplied

- ✓ Distilled or deionised water for dilution of the Wash buffer concentrate.
- ✓ Appropriate equipment for pipetting, liquid dispensing and washing.
- ✓ Spectrophotometer/colorimeter (microplate reader – wavelenght 450 nm).

Precautions for Use

- ✓ Safety Precautions
- All ingredients of the kit are intended for laboratory use only.
- The samples of cerebrospinal fluid, IgG 1, IgG 2 and IgG-biot should be regarded as contagious and

handled and disposed of according to the appropriate regulations. Autoclave all reusable materials that were in contact with the samples of cerebrospinal fluid for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramines for 30 min.

- Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

✓ Handling Precautions

- Avoid contamination of samples and kit reagents.
- Avoid cross-contamination of reagents.
- Follow the assay procedure indicated in the Instruction manual.
- Variations in test results are usually due to:

  * Insufficient mixing of reagents and samples
  * Inaccurate pipetting and inadequate incubation times in the assay procedure
  * Poor washing technique or spilling the rim of well with sample or other reagents
  * Use of identical pipette tip for different solutions

Assay Protocol

Reagent Preparation

- ✓ Allow all kit components to reach room temperature.
- ✓ Prepare Wash buffer by diluting the concentrate 10 times with an appropriate volume of distilled or deionised water (100 mL of the concentrated Wash buffer + 900 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 to 37°C in a water bath. Diluted Wash buffer is stable for one week if stored at 2 to 10°C.
- ✓ Do not dilute 4-nitrophenyl phosphate buffer and Dilution buffer, they are ready to use.

Sample Preparation

- ✓ Before using dilute samples of cerebrospinal fluid 1:1 in dilution buffer – e.g. 100 µL of sample of cerebrospinal fluid + 100 µL of dilution buffer.
- ✓ Before using dilute mouse anti-human IgG antibody 1 (IgG 1) and mouse anti-human IgG antibody 2 (IgG 2) 1:1 100 times in dilution buffer and vortex – e.g. 0.1 mL IgG 1 + 0.1 mL IgG 2 + 9.8 mL of dilution buffer. (*Note: For one microtitre plate you will need approx. 10 mL of diluted mixture of IgG 1 and IgG 2.*)
- ✓ Before using dilute rabbit anti- mouse IgG antibody with biotin (anti-IgG-biot) 100 times in dilution buffer (DB) and vortex – e.g. 0.1 mL IgG-biot + 9.9 mL of dilution buffer. (*Note: For one microtitre plate you will need approx. 10 mL of diluted IgG-biot.*)
- ✓ Before using dilute alkaline phosphatase marked by streptavidin 100 times in dilution buffer and vortex – e.g. 0.1 mL Str-AP + 9.9 mL of dilution buffer (DB). (*Note: For one microtitre plate you will need approx. 10 mL of diluted Str-AP.*)
- ✓ Rehydrate dried powder NF-L antigen with Dilution Buffer with volume stated on a vial. Final concentration of NF-L is 100 ng/mL. For future use the NF-L concentrate, store at -20°C, maximum 6 months. Before used the Standards NF-L are received by dilution of NF-L concentration (100 ng/mL) in Dilution Buffer by the following tables:

Table 1 Scheme of preparation of Standards (ST A –ST F):

Volume of Standard	Volume of dilution buffer in µL	Final concentration of Standard in pg/ml	Sign of Standard
		100 000	**NF-L**
25 µL NF-L	980 µL	2000	**ST F**
500 µL ST F	500 µL	1000	**ST E**
500 µL ST E	500 µL	500	**ST D**
500 µL ST D	500 µL	250	**ST C**
400 µL ST C	600 µL	100	**ST B**
400 µL ST B	400 µL	50	**ST A**

Assay Procedure

1. Allow the antigen coated strips to reach room temperature before opening in order to prevent water condensation within the wells. Withdraw an adequate number of antigen coated strips (colourless). Put the remaining strips back in the aluminium pouches and seal them if possible, keep the desiccant inside.
2. Wash and aspirate the wells three times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
3. Pipette 100 µL of diluted Standards (ST A – ST F) and diluted samples of cerebrospinal fluid to the wells according to the pipetting scheme in Plate Layout. It is necessary to fill two wells of parallel strips by one sample (e.g. well "a" of strip 1 as well as well "a" of strip 2), because strips 2, 4, 6, 8, 10, and 12 are controls (backround) of strips 1, 3, 5, 7, 9, and 11.
   Fill the first well of strips 1 and 2 with Dilution buffer to estimate the total reaction background. Fill the next wells of strips 1 and 2 with diluted Standards NF-L (ST A – ST F). Fill the remaining wells with the diluted samples of cerebrospinal fluid (S1, S2, S3,...) in doublets (parallel strips). Applying samples in doublets of one strip minimize the laboratory error. Incubate for 2 hours (± 5) minutes at 37°C.
4. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (*see Safety Precautions*). Wash and aspirate the wells five times with 250 µl/well of Wash buffer as in step "2".
5. Add 100 µL of the diluted mixture of IgG 1 and IgG 2 into each well.
6. Incubate for 60 (± 5) minutes at 37°C.
7. Aspirate and wash the wells five times with 250 µl/well of Wash buffer as in step "2".
8. Dispense 100 µL of the diluted antibody anti-IgG-biot into each well.
9. Incubate for 60 (± 5) minutes at 37°C.
10. Aspirate and wash the wells five times with 250 µl/well of Wash buffer as in step "2".
11. Add 100 µL of the diluted Str-AP into each well.
12. Incubate for 30 (± 2) minutes at 37°C.
13. Aspirate and wash the wells five times with 250 µl/well of Wash buffer as in step "2".
14. Pipette 100 µL of 4-nitrophenyl phosphate buffer into each well.
15. Incubate for 60 (± 5) minutes at room temperature. Cover the strips with an aluminium foil or keep them in the dark during the incubation with 4-nitrophenyl phosphate buffer.
16. Read the absorbance at 405 nm with a microplate reader within 5 minutes.

Data Analysis

Calculation of Results

✓ Processing of Results

Calculate concentration of NF-L for samples of cerebrospinal fluid.

1. First subtract the absorbencies (OD) of the wells of even numbers (the wells number 2,4,6,8,10...) from the absorbencies (OD) of the wells of odd numbers (the wells number 1,3,5,7,9,...) – e.g. well 1A – 2A, well 1B – 2B etc.
2. Then subtract the absorbance (OD) of the background (absorbance of the Dilution Buffer well) from the absorbencies (OD) obtained in the step "1".
3. Construct the calibration curve by plotting the absorbance (Y) of Standards (ST A – ST F) versus the known concentration (X) of Standards (in Table 1, Scheme of preparation of Standards).
4. Calculate the concentration of NF-L for samples of cerebrospinal fluid by equation of calibration curve. Use the value of sample absorbance (OD) instead of the value y. The values x represent the concentration of NF-L for samples of cerebrospinal fluid. Note that you need to use the diluting factor in the calculation (i.e. if you dilute sample of cerebrospinal fluid 1:1, you have to multiply the final concentration of NF-L by 2).

Performance Characteristics

✓ Validity of the test

The test is valid if:

✓ The mean absorbance (OD) of Standard A (ST A) is less than 0.200.

✓ The mean absorbance (OD) of Standard F (ST F) is more than 1.500.

✓ The mean absorbencies (OD) of Standards (ST A – ST F) can be lined up as follows:

ST A < ST B < ST C < ST D < ST E < ST F

✓ Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) evaluation was performed with samples of variable absorbance values.

1. Variability (intraassay) (n = number of parallels of the same microtitration platen):

n	A	±δ	min – max	CV repro.
16	0.938	0.077	0.806 – 1.075	8.2 %
16	0.576	0.047	0.49 – 0.653	8.2 %

2. Reprodukovatelnost (interassay) (n = number of an independent examination of the same sample):

n	A	±δ	min – max	CV repro.
8	0.324	0.017	0.299 – 0.347	5.4 %
8	0.397	0.034	0.346 – 0.444	8.6 %
8	0.688	0.026	0.655 – 0.731	3.8 %

Resources

References

- ✓ W.J.A. Van Geel, L.E. Rosengren, M.M. Verbeek: An enzyme immunoassay to quantify neurofilament light chain in cerebrospinal fluid, Journal of Immunological Methods 296 (2005), 179-185.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution buffer	Dilution buffer	M2	M2								
B	STA	STA	M3	M3								
C	STB	STB	M4	M4								
D	SYC	SYC	M5	M5								
E	STD	STD	M6	M6								
F	STE	STE	M7	M7								
G	STF	STF										
H	M1	M1										