Human Neurofilament, Light Polypeptide (NEFL) ELISA
Cat No: K12-4364

Principle:
The Human Neurofilament, Light Polypeptide ELISA is sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Neurofilament, Light Polypeptide in samples. Standards or Samples are added to the microtiter well which is pre-coated with Human Neurofilament, Light Polypeptide monoclonal Antibody. Biotinylated Human Neurofilament, Light Polypeptide antibody is added to the microplate to form a complex. Subsequently Streptavidin-HRP conjugate is pipetted. After incubation and a washing step TMB Substrate A and B, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Human Neurofilament, Light Polypeptide in the samples is directly proportional to the yellow color developed in the wells.

Intended Use:
This Kit is used to assay the level of Human Neurofilament, Light Polypeptide in human serum and plasma samples. The Kit is For Laboratory / Research Use Only.

Materials provided in the Kit:
1. Anti-Human Neurofilament, Light Polypeptide Coated Microtitre Plate (96 wells) – 1 no
2. Biotinylated Human Neurofilament, Light Polypeptide Antibody – 1 ml
3. Human Neurofilament, Light Polypeptide Standard (concentrated, 40 ng/ml) – 0.5 ml
4. Streptavidin-HRP Conjugate – 6 ml
5. (30X) Wash Buffer – 20 ml
6. Standard Diluent– 3 ml
7. TMB Substrate A – 6 ml
8. TMB Substrate B – 6 ml
9. Stop Solution – 6 ml
10. Instruction Manual

Materials to be provided by the End-User:
1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

Storage Information:
1. All reagents should be stored at 2ºC to 8ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:
1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Specimen Collection and Handling:
Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.
1. The kit cannot test samples which contain NaN_3, because NaN_3 inhibits HRP activity.

2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
4. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):
1. Bring all reagents to Room Temperature prior to use.
2. To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

Procedural Notes:
1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human Neurofilament, Light Polypeptide. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Neurofilament, Light Polypeptide present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Human Neurofilament, Light Polypeptide concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Neurofilament, Light Polypeptide.
4. It is recommended that all Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to poor sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:
1) Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.
2) Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and Standard Diluent.

Standard Concentration	Standard No	Dilution Particulars
40 ng/ml	Standard, concentrated	Original Standard provided in the Kit
20 ng/ml	Standard No.5	120 ul Original Standard + 120 ul Standard diluent
10 ng/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard diluent
5 ng/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard diluent
2.5 ng/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard diluent
1.25 ng/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard diluent

** refer accompanying sheet with the Standard, concentrated in the kit*

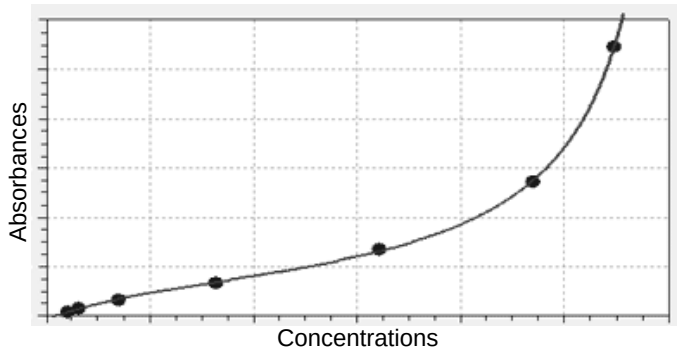
3) The quantity of the plates depends on the quantities of samples and standards to be tested. It is suggested to remove the number of strips required for the assay.
4) Pipette **50 ul** of **Standards** and **40 ul Samples** into the respective wells as mentioned in the work list.
Note do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.
5) Pipette **10 ul** of **Biotinylated Human Neurofilament, Light Polypeptide Antibody** into each sample well.
Do not pipette into the blank and standards wells.

6) Pipette **50 ul** of **HRP Conjugate** into each sample and standards well.
Do not pipette into the Blank well.
7) Cover the plate and incubate for 1 hour at 37°C in the incubator.
8) Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping the plate on an absorbent paper. Wipe off any liquid from the bottom of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
9) Add **TMB Substrate A 50 ul** and **TMB Substrate B 50 ul** respectively to each well. Gently mix.
10) Incubate for 10 min at 37°C in dark.
11) Pipette **50 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
12) Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution. Blank the zero standard for net absorbance.

Calculation of Results:
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Typical Graph



Precautions:
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:
Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to **0.116 ng/ml.**

Specificity:
The antibodies used in the kit for capture and detection are specific for human Neurofilament, Light Polypeptide.

Assay Range:
1.25 ng/ml to 20 ng/ml

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The Linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human Neurofilament, Light Polypeptide and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum (n=5)	85-105%	86-109%	83-112%
EDTA plasma (n=5)	84-106%	85-117%	83-118%
heparin plasma (n=5)	83-99%	80-95%	82-93%

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ASSAY PROCEDURE

1	Bring all reagents to room temperature before use.		
2	Pipette Standards 1 - 6 Samples	50 ul	40 ul
3	Pipette Human Neurofilament, Light Polypeptide Biotin Detection Antibody		10 ul
4	Pipette Streptavidin :HRP Conjugate	50 ul	50 ul
5	Incubate	60 minutes (37ºC)	
6	1X Wash Buffer	Decant, 4 x 300 ul	
7	Pipette Substrate (A)	50 ul	50 ul
8	Pipette Substrate (B)	50 ul	50 ul
9	Incubate in the dark	10 minutes (37ºC)	
10	Pipette Stop Solution	50 ul	50 ul
11	Measure 450 within 15 mins		