Human Neurofilament (NF)ELISA Kit

Cat No. MBS264177

This product is suitable for the in vitro quantitative detection of Human serum, plasma or cell culture supernatant and organizations in the natural and recombinant NF concentration. For detection of other special sample types please contact our technical support. The kit is for Research Use Only. Please read the instructions carefully before using and confirm all kit components are included.

This kit employs the "Double Antibody Sandwich" technique. The principle of Double Antibody Sandwich is based on the characteristics of a target analyte with more than two possible epitopes which can be identified by both the pre-coated capture antibody and the detection antibody simultaneously. The process is as follows:

1. Pre-coat antibodies to the plate, and then, via washing, remove all antibodies and impurities that did not bind to the plate. The remaining sites on the plate are blocked with irrelevant proteins.
2. Once remaining plate sites have been blocked, sample containing the target analyte can be added, which will result in the target analyte becoming immobilized by the analyte-specific capture antibodies, forming an antigen-antibody complex. The wells are then washed to remove all unbound particles and impurities.
3. A biotin-labeled antibody is then added to the wells that is also specific for the target analyte, resulting in an antibody-antigen-antibody complex. The plate is again washed to remove unbound antibodies and impurities.
4. Next, horseradish peroxidase + avidin is added to the wells and binds with the biotin-labeled antibodies. The quantity of reporter enzyme is now positively correlated to the quantity of target analyte in the sample. The wells are then washed again to remove any impurities.
5. Finally, substrates for the HRP reaction are added, and the sample concentrations can then be computed/calculated from the resulting coloration changes.

Note: Antibodies can be labeled with multiple biotin molecules, which can bind to HRP-Avidin and result in multiple HRP complexes per antibody. This tends to show higher sensitivity and amplification effects compared to traditional direct HRP-Antibodies.

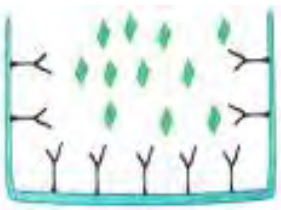
【Detection principle of Human Neurofilament (NF) ELISA kit】

As mentioned above, this kit utilizes the Double Antibody Sandwich ELISA technique. The pre-coated antibody is an anti-Human NF monoclonal antibody, while the detection antibody is a biotinylated polyclonal antibody. Samples and biotinylated antibodies are added into ELISA plate wells and washed out with PBS or TBS after their respective additions to the wells. Then Avidin-peroxidase conjugates are added to the wells in after. TMB substrate is used for coloration after the enzyme conjugate has already been thoroughly washed out of the

wells by PBS or TBS. TMB reacts to form a blue product from the peroxidase activity, and finally turns to yellow after addition of the stop solution (Color Reagent C). The color intensity and quantity of target analyte in the sample are positively correlated.

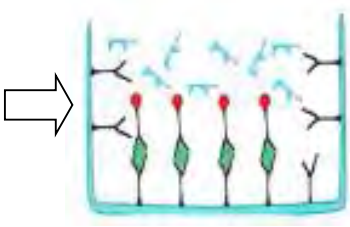
General Schematic of the Double-Antibody Sandwich Principle:

The first step

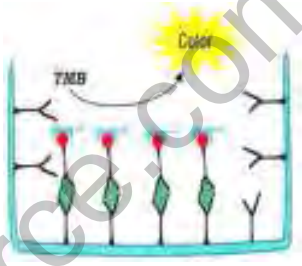


The second step

The third step



The fourth step



【Kit Components】

Name	96 Tests	48 Tests	Storage
Pre-coated plate	8×12	8×6	4/-20℃
Human NF Standards	2 vial	1 vial	4/-20℃
Biotinylated antibody (1:100)	1vial	1 vial	4/-20℃
Enzyme conjugate(1:100)	1vial	1 vial	4/-20℃
Enzyme diluent	1vial	1 vial	4/-20℃
Antibody diluent	1vial	1 vial	4/-20℃
Standard diluent	1vial	1 vial	4/-20℃
Sample diluent	1vial	1 vial	4/-20℃
Washing buffer (1:25)	1vial	1 vial	4/-20℃
Color Reagent A	1vial	1 vial	4/-20℃
Color Reagent B	1vial	1 vial	4/-20℃
Color Reagent C	1vial	1 vial	4/-20℃
Manual	1 set	1 set	RT

Notes：

RT：Room temperature

Standard：Lyophilized

Color Reagent A: Avoid light

【Materials Required But Not Provided With Kit】

1. Microplate reader (450nm detection wavelength filter, with optional 570nm or 630nm correction wavelength filters).

2. Washer (adjustable injection volume to ensure that each well receives 350µl without overflow).
3. Clean benches, biological safety cabinets, fume hoods.
4. High-precision single-channel pipette (range 0.5-10µl-20µl, 20-200µl, 200-1000µl).
5. High-precision multi-channel pipette (8 or 12, the range of 50-300µl of).
6. 37℃ incubator.
7. Low temperature centrifuge.
8. Refrigerators (4℃, -20℃, -86℃).
9. Analytical balance.
10. Scissors, tweezers, pliers, etc.
11. Plate mixer, low-frequency oscillator, etc.

【Additional Materials Required】

1. Centrifuge tubes (capacity of 1.5ml, 5ml, etc.).
2. Disposable pipette tips (range of 0.5-10µl-20µl, 20-200µl, 200-1000µl).
3. Pure water or distilled water.
4. Coordinate paper.
5. Absorbent paper.

【Sample collection Note】

1. Blood collection tubes should be both pyrogen- and endotoxin-free.
2. Hemolyzed or hyperlipidemic specimens are not recommended to be used.
3. Samples ultimately should appear clear and mostly transparent. All particulates should be removed through centrifugation.
4. If collected samples are not used immediately, they should be divided according to single usage quantities and stored frozen at -20-80℃, carefully avoiding repeated freeze-thaw cycling.
5. Sample dilution optimization is often necessary for proper sample resolution within the standard curve. Pre-experiments are always recommended to be performed prior to running the bulk of the samples, in order to determine if an optimizing dilution should be made to the samples.
6. It is recommended to collect sufficient samples for running multiple tests, since sometimes some users can experience issues that may result in a loss of data.
7. Always wear protective outerwear while collecting/handling samples (e.g. wearing gloves, coat, respirator, etc.) and be aware of all potential risks involved in handling your specimens.
8. Specimen processing should be conducted inside a properly-maintained biological safety cabinet.

【Sample Preparation】

1. Serum: Place collected whole blood in refrigerator at 4℃ overnight. Then centrifuge for 10min at 1000-3000rpm. Take supernatant and either test immediately or place samples at -20℃/-80℃(1-3 months) for storage.
2. Plasma: Take plasma where EDTA, sodium citrate, or heparin has been added as

anticoagulant. Mix well. Centrifuge mixture for 10min at 1000-3000rpm. Take supernatant and test immediately or place samples at -20℃ /-80℃ (1-3 months) for storage.

3. Tissue homogenate: Take tissue slices which have been washed in 0.01M PBS and then a tissue protein extraction reagent has been added according to proportion of 1g to 5-10mL and then mixed on ice bath. After sufficient homogenization, please then centrifuge for 10min at 5000-10000rpm. Take supernatant for immediate testing, or place samples at -20℃/-80℃ (1-3 months) for storage.
4. Cell culture: Centrifuge for 10min at 1000-3000rpm. Take supernatant for immediate testing, or place samples at -20℃/-80℃ (1-3 months) for storage.
5. For urine, ascites, cerebrospinal fluid, etc: Centrifuge for 10min at 1000-3000rpm. Take supernatant for immediate testing, or place samples at -20℃/-80℃ (1-3 months) for storage.

Note: It is recommended that the user be well-informed regarding the general range of concentrations that are expected for their samples. We would suggest consulting the scholarly literature for references to similar samples' concentrations, and then to consider diluting your samples accordingly.

【Note】

1. The re-dissolved standard cannot be stored again once prepared, so please do not attempt to re-freeze it once it has been reconstituted.
2. Due to shaking/inversion during transport, centrifuging of the tubes/bottles of the kit might be necessary to consolidate the material contained within. Tubes should be shaken manually or centrifuged for 1 min at 1000rpm to pool all material to the bottom.
3. Concentrated washing buffer might crystallize slightly. Use a water bath to help the dissolution during diluting process. The crystals must be totally dissolved when preparing the washing buffer.
4. The prepared standard is intended to only be a single-use aliquot, so please do not try to re-use the standard that has already been tested. Please use the second vial provided if you run the assay again.
5. Only use reagents/components that came directly with this kit. Do not mix batches/lots from other orders of this kit, or from different kits.
6. Ensure the reagents are well mixed. For the reagents in the microplate, adequate mixing is particularly important for accurate test results. It is recommended to employ a micro-oscillator (at the lowest frequency). If a micro-oscillator is not available, please slightly shake the microplate manually for 1 min, in a circular motion in order to make sure the wells are sufficiently mixed.
7. Please ensure the kit has been brought to room temperature prior to beginning the assay.
8. Standards are always recommended to be tested in duplicate or triplicate.
9. Place the unused microplate strips into the foil bag at 2-8℃ for storage (if you intend to use the strips within a relatively short timeframe).
10. The chromogen reagent is sensitive to light, therefore please avoid exposing to light.
11. Kits that have passed their expiration date should not be used.
12. When using dual-wavelength, the wavelengths should be set at 450nm and 630nm.

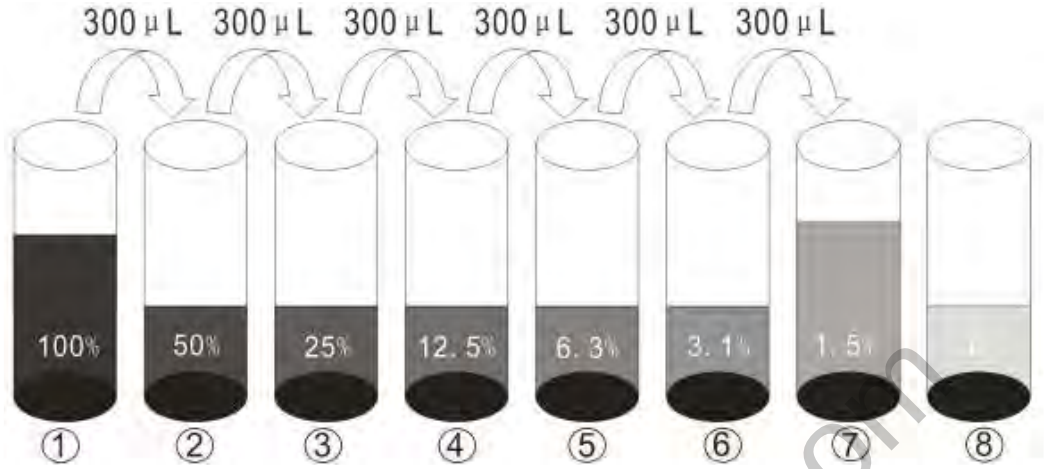
13. All the samples, washing buffer and wastes should be treated as biowaste. Color Reagent C is 1M sulfuric acid, so please pay close attention to safety when it is used.
14. Sample addition should always be done via pipette or similar instrument. Calibrate the instrument prior to running the assay in order to avoid experimental errors. Please add samples to the wells quickly, as it is recommended to control the sample addition time to less than ~5 minutes. You might want to consider multi-pronged pipettes if this helps with the loading time.
15. Do not re-use the adhesive strips. Cut them to size if you only use part of the plate at a time. Always discard after use.
16. New standard curves should be made for every new run of the assay. If the observed concentrations of test samples are too high (OD value of the sample is higher than that of standard well maximum concentration), dilute by a certain factor, and correct for said factor in the end calculations.
17. Samples containing NaN3 cannot be tested due to NaN3 inhibiting the activities of horseradish peroxidase (HRP).
18. When washing plate via plate washer, the volume of buffer injected into each well should be slightly more than 350μl. Make sure the sampling head is not jammed or blocked. Also, if washing by hand, please take care when using an absorbent material to remove excess water – make sure this absorbent material wasn't used to clean any of the other reagents to prevent contamination.
19. After the coloration reaction termination by Color Reagent C, please read OD within 10 minutes.
20. If duplicate wells were performed, the mean value of the wells should be used.
21. Hemolyzed samples may cause false positive results, so we consider these samples to be incompatible with this kit.
22. During the assay, please try to control the humidity to ~60%.
23. We recommend regularly checking the thermostat and calibration in order to confirm the incubation temperature remains at a stable 37℃.
24. For the 48T ELISA Kit, all components are 50% the amount of those in the 96T.

【Test preparation】

1. Please remove ELISA Kit from refrigerator 20 minutes in advance, and begin test once it has been brought to room temperature.
2. Dilute the concentrated washing buffer with double distilled water (1:25). Return unused quantity back to the box.
3. Standard: Add 1.0ml Standard Diluent to lyophilized standard vial and allow to sit for 30 min. After the standard has completely dissolved, mix it slightly and mark with a label on the tube. It is recommended to use the following concentration values for the standard curve: 20,10, 5, 2.5, 1.25, 0.625, 0.312ng/mL. Note: Make absolutely sure the lyophilized standard completely dissolved and well mixed.
4. Legend of standard sample dilution method: Take 7 clean tubes and label them with their expected concentrations (10,5, 2.5, 1.25, 0.625, 0.312, , 0ng/mL). Add 300μL Standard Diluent into each tube. Pipette out 300μl diluent from the reconstituted standard and add to the tube labeled 10ng/mL and mix well. Further Pipette out 300μl diluent from the

10ng/mL tube, and add to the 5ng/mL, and mix well. Repeat these steps through the 0.312ng/mL standard. Standard Diluent in the 0ng/mL tube is the negative control.

Note: The reconstituted standard solution（20 ng/mL）should be discarded after running the assay – it is not reusable.



Note: Reconstituted standard stock solution cannot be reused.

5. Biotinylated Antibody: Remove the appropriate volume of Biotinylated Antibody solution for the quantity of wells intending to be assayed, and dilute with Antibody Diluent in a proportion of 1:100. This should be prepared 30min in advance, and we would absolutely recommend not re-using for additional assaying.
6. Enzyme Conjugate: Remove the appropriate amount of Enzyme Conjugate solution for the quantity of wells intending to be assayed, and dilute with the Enzyme Diluent in a proportion of 1:100. This should be prepared 30min in advance, and we would absolutely recommend not re-using for additional assaying.
7. Color Reagent: Prepare Color Reagent solution 30min in advance by adding Color Reagent A and Color Reagent B by the proportion of 9:1.

【Washing method】

1. Automatic plate-washing: The required amount of wash buffer is 350μl, and the injection and extraction intervals should be ~20－30secs. Please be well aware of the operation before putting the machine into practice.
2. Manual plate-washing: add 350μl wash buffer to each well and let stand for 30sec. Shake plate to remove as much liquid as possible, and dab the plate with absorbent paper if necessary. During the plate-washing process, please pay close attention to the wash buffer-adding steps to avoid contamination and well-jumping.

【Steps】

1. Remove number of strips desired, and allow to acclimate to room temperature. The unused strips and desiccant should be placed back into the sealed aluminum foil bag and stored at 2-8°C.
2. Set aside blank wells (if measuring at dual-wavelength, the blank wells can be ignored)
3. Add standards or samples to their corresponding wells (100μL for each well). Please

remember that the 0ng/mL well should be 100μL of Standard Diluent. Seal the wells/plate with the adhesive tape strip, and incubate at 37℃ for 90min.

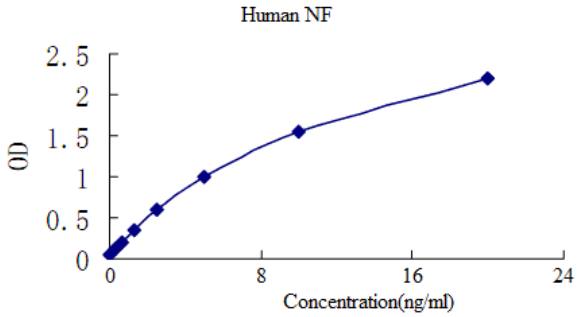
4. Prepare required quantity of Biotinylated Antibody 30min in advance.
5. Wash ELISA plate 2 times
6. Add prepared Biotinylated Antibody to each well (100μL per well). Seal reaction wells with adhesive tape strip, and incubate at 37℃ for 60min.
7. Prepare required quantity of Enzyme Conjugate 30min in advance.
8. Wash ELISA plate 3 times
9. Add prepared Enzyme Conjugate to each well other than the blank wells (100μl for each). Seal the wells with the adhesive tape strip, and incubate at 37℃ for 30min.
10. Wash ELISA plate 5 times.
11. Add 100μL of the prepared Color Reagent to individual wells (also into blank well), incubate protected from light at 37℃. When the coloration of the highest standards become darker, and the color gradient appears, the incubation can be stopped. The chromogenic reaction should be controlled to within 30 min.
12. Add 100μL Color Reagent C to each individual well (also into blank well). Mix well. Read OD（450nm）within 10 min.

【Result determination】

1. The OD values of each sample and standard should have the values of the blank well subtracted.
2. Draw standard curve manually. Take the concentration values of standards as X- and OD readings as the Y-coordinates. Use a smooth line to connect each coordinate point of the standard values. The concentration of samples can be found by inputting the sample OD values into the line equation for the standard curve. It is recommended to employ professional curve software (e.g. curve expert 1.3) to analyze and compute the results.
3. If the sample OD is higher than that of the highest standard in standard curve, the sample should be diluted (or diluted further) and the assay reran. Multiply the results by the dilution factor when calculating for the unknown.

Note: This chart is only for reference. Calculation of user sample content should always be subject to the standard curve made during the same experiment

【Reference curve】



Note: Again, please not that his chart is for reference only, and that user sample concentrations should be based on the standard curve generated at the same time as their samples on the same plate.

【Summary of operating procedures】

step	Summary of operating procedures
1	Prepare reagents, samples and standards.
2	Add the prepared samples and standard & incubate at 37 ℃ for 90 minutes.
3	Wash 2x, add Biotinylated Antibody solution & incubate at 37 ℃ for 60 minutes.
4	Wash 3x, then add the Enzyme working solution & incubate at 37 ℃ for 30 minutes.
5	Wash 5x, then add the Color Reagent solution & incubate at 37 ℃ up to 30 minutes.
6	Add the Color Reagent C.
7	Use microplate reader to measure OD within 10 minutes of adding Color Reagent C.
8	Calculate the content of samples being tested

【Detection range】20 ng/mL-0.312 ng/mL
【Sensitivity】the minimum detectable Human NF up to 0.06 ng/mL.
【Intra assay Precision】≤ 8%
【Inter assay Precision】≤ 12%
【Recovery】70 - 110 percent.
【Storage】-20℃ [Short-term should be stored at 4℃(such as ~2 weeks)]
【Uses】used for in vitro quantitative analysis of liquid medium samples (Universal).
【Specifications】96T.
【Production Date】See microtiter plates aluminum foil bag sealing stamp.
【Validity】12 months (-20℃).
【Reference】

1. Löhrke, S; Brandstätter, JH; Boycott, BB; Peichl, L (Apr 1995).
2. Alberts, Bruce (2002).
3. Lalonde R, Strazielle C; Strazielle (2003).