

(For scientific research use only, not for clinical diagnosis!)

Human Heavy Peptide Neurofilament

Protein (NEFH) Quantitative Detection

Kit (ELISA) Instructions for Use

Specification: 96T/48T Catalog Number:

SYP-H3036

Purpose: Used to detect human heavy peptide neurofilaments in serum, plasma, cell culture supernatant and other samples

The concentration of protein (NEFH).

Please read the instructions carefully before use. If you have any questions, please contact us via:

Official hotline: 400-999-8863

Technical phone number: 18358180525

Email: UpingBio@163.com

Company website: www.upingbio.com For specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life. When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

[Kit performance]

Physical properties: Each liquid component is clear and transparent, with no sediment or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Calibration curve linearity: The correlation coefficient r value of the calibrator dose-response curve is greater than or equal to 0.9900.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%. **Sensitivity:** The lowest detectable dose is less than 7.812 pg/mL.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes the natural human heavy peptide neurofilament protein (NEFH) and has no crossover with structural analogs.

Stability: Stored at 2°C-8°C, validity period is 6 months.

Detection range: 31.25 pg/mL – 1000 pg/mL.

【Experimental principle】

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-human heavy peptide neurofilament protein (NEFH) antibody (solid-phase antibody), add human heavy peptide neurofilament protein (NEFH) calibrator and the sample to be tested, and then add biotin labeling. After the antibody is incubated and fully washed, HRP-coupled avidin is added. After incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen-biotin is formed on the solid surface of the microplate. Labeled antibody-avidinase sandwich complexes. Add TMB chromogenic solution to produce a blue product. Under the action of the stop solution, it is finally converted into yellow. The absorbance (OD value) is measured on a microplate reader at a wavelength of 450nm. The absorbance (OD value) is related to the human heavy peptide nerve in the sample to be tested. The concentration of silk protein (NEFH) is positively correlated. By fitting the calibrator curve, the concentration of the human heavy peptide neurofilament protein (NEFH) in the sample can be calculated.

[Kit components and storage]

Components		quantity	Main
Calibrator	High Standard	2 vial	Calibrator freeze-
Calibration solution	Reconstitution	2 vial	PBS
Calibrators & Sample Diluents	Standard & Sample Diluent	25mL	PBSTN
coated microplate	Microelisa Stripplate	96T/48T	Pre-coated solid phase
biotin antibody	Bio-Antibody	10mL	biotin antibody
HRP labeled avidin	HRP- Conjugate	10mL	HRP labeled avidin
TMB chromogenic	TMB	10mL	TMB
stop solution	Stop Solution	6mL	acidic solution
20×concentrated	20X Wash Solution	25mL	0.05%Tween20
manual	manual	1 serving	--
Ziplock bag	Ziplock bag	1	--
self-adhesive	self-adhesive	4 pieces	--

Note: 1. Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2. The test kit should be stored at 2-8°C. Expired test kits must not be used.
3. If the coated microplate is not used up in a single time, remember to seal it and store it at 2-8°C.
4. The reconstituted calibrator can only be used on the same day.

5. If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use.

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Supervision phone number:
10150115141

**Prepare your own test equipment required for the
test (not provided, but can assist in purchasing) 1.**

Standard specification microplate reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

[Kit limitations]

1. For scientific research use only and not
for clinical diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest calibrator concentration value,
please dilute the sample appropriately and then re-measure. 6. Human anti-mouse

and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

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【Precautions】

1. This kit is for in vitro research only and not for clinical diagnosis.
2. Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.
3. Incubate strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.
4. Incorrect plate washing may lead to inaccurate results. Be sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.
5. Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
6. The substrate chromogenic solution should be colorless or very light in color.
7. Avoid cross-contamination of reagents and specimens to avoid erroneous results.
8. Avoid direct exposure to strong light during storage and incubation.

9. After balancing to room temperature, open the sealed bag to prevent water droplets from condensing on the cold slats.

10. Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.

11. The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of $450\pm 10\text{nm}$.

Optical density ranges from 0-3.5. It is recommended to preheat 15 minutes in advance before use.

12. Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources.
13. The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.
14. Do not use expired reagents.

【Sample preparation and storage】

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant: centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum: Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma: Anticoagulate with heparin, citrate or EDTA, and centrifuge at $2000 \times g$ for 20 minutes at 2-8°C within 30 minutes of blood drawing. To eliminate the influence of platelets, it is recommended to further centrifuge at $10,000 \times g$ for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis medium: For adherent cells, remove the culture medium and replace with PBS, physiological saline or blood-free

Wash with culture medium. Add an appropriate amount of lysis solution and pipet several times to ensure full contact between the lysis solution and cells.

Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them once with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, pipet with a pipette to disperse the cells, and flick with your fingers to fully lyse the cells.

After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine: Collect in sterile tubes and centrifuge at 2000×g for 20 minutes.

Carefully collect the supernatant. If a precipitate forms, centrifuge again.

【Reagent preparation】

1. Before use, all components must be rewarmed for at least 120 minutes to ensure full rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating

in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

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[Calibration dilution method]

Steps for redissolving the calibrator: Redissolve the calibrator with the calibrator reconstituted solution. Add all the liquid in one bottle of the calibrator reconstituted solution to the bottle of calibrator freeze-dried powder. Vortex gently to ensure thorough mixing. , the mother solution concentration of the calibrator after redissolution is 2000 pg/mL , mix thoroughly before dilution.

Dilution steps of the calibrator mother solution: Let the calibrator working solution stand for 1-2 minutes before dilution. Use the calibrator & sample universal diluent to double dilute the calibrator mother solution. The doubling dilution method: take 7 EP tubes, and Add 500 μ L calibrator & sample diluent, pipet 500 μ L from the 2000 pg/mL calibrator mother solution into the first EP tube and mix to prepare a 1000 pg/mL calibrator working solution. Follow this step in sequence. Pipette and mix. As shown on the following page.



2000 pg/mL

Recommended dilution concentration of calibrator: It is recommended to prepare the following concentrations: 1000, 500, 250, 125,

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62.5, 31.25, 0 pg/mL, and used as the calibrator concentration value of the fitting standard curve.

Tip: Add the calibrator & sample universal diluent directly to the last tube as the 0 value. There is no need to draw liquid from the penultimate tube. The diluted calibrator working solution needs to be prepared and used immediately.

【Operating Procedure】

Recommended sample dilution scheme: It is recommended that teachers conduct preliminary experiments to explore the optimal dilution ratio of samples before conducting formal experiments.

All reagents and components should be returned to room temperature first. It is recommended to perform duplicate wells for calibrators, quality control materials and samples.

1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.
2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Set up the calibrator hole, sample diluent hole, blank hole and sample hole. Add 50 μ L of calibrator of different concentrations to each of the calibrator holes. Add 50 μ L of sample diluent to the sample diluent hole. Do not add it to the blank hole. Add the sample hole to be tested. Sample 50 μ L. Except for the blank wells, add 100uL of biotin antibody to each well, cover the reaction plate with a sealing film, and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

4. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, and let it sit.

1min，甩去洗涤液，吸水纸上拍干，如此重复 5 次。若使用自动洗板机，请按洗板机操作程序进行洗板，添加浸泡 30s 的程序，可以提高检测的精度。洗板结束，加底物前，要在干净不掉屑的纸上，充分拍干反应板。

5、除空白孔外，每孔加入 HRP 标记亲和素 100uL，用封板膜盖住反应板，37°C水浴锅或恒温箱避光温育 20min。

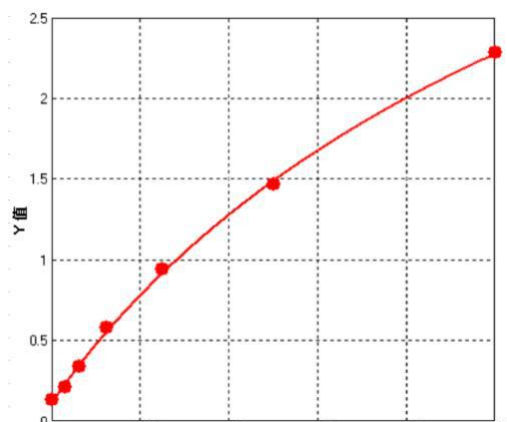
6、重复步骤 4。

7、所有孔中加入 TMB 显色液 100μL。用封板膜盖住反应板，37°C水浴锅或恒温箱避光温育 15min。

8、所有孔加入终止液 50μL，在 450nm 波长酶标仪上读取各孔吸光度（OD 值）。

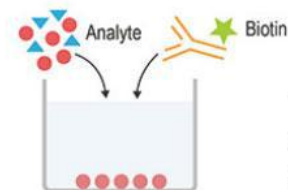
【结果计算】

1、以校准品浓度做为横坐标，对应的吸光度（OD 值）作为纵坐标，利用计算机软件，采用四参数 Logistic 曲线拟合（4-pl），创建标准曲线方程，通过样本的吸光度（OD 值），利用方程计算样品的浓度值。【用 ELISA Calc 软件计算】2、如果样品被稀释，通过上述方法测得的浓度值，要乘以稀释倍数，才是样品的最终浓度。



（示意图，仅供参考）

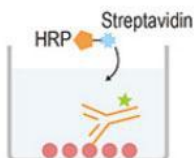
【操作概要】



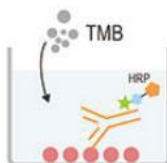
1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液, 37°C孵育60分钟。



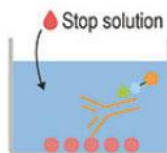
2、弃掉板内液体, 洗板5次。



3、每孔加入100uL HRP酶结合物工作液 37°C孵育20分钟, 弃掉板内液体, 洗板5次。



4、每孔加入100uL TMB显色液, 37°C孵育 15分钟。



5、每孔加入 50uL 终止液。



6、立即在 450nm波长下读数, 处理数据。

【问题分析】

问题描述	可能原因	相应对策相应对策
阴阳性对照 结果不稳定	吸液或加液不准	检查移液器及吸头
	平衡时间太短	保证充足的平衡时间
	洗涤不完全	保证洗涤时间和洗涤次数 及每孔的加液量
显色很弱或 无色	孵育时间太短	保证充足的孵育时间
	实验温度不正确	使用推荐的实验温度
	试剂体积不够或漏加	检查吸液及加液过程， 保证 所有试剂按顺序
	稀释不正确	
	酶标记物失活或底物失效	混合酶结合物和底物， 通过 迅速显色来检查
读数数值低	酶标仪设置不正确	Check the wavelength and filter settings on the
		Turn on the microplate
Large coefficient of	Adding fluid incorrectly	Check the filling
High background value	The working	Use the recommended
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check
	The lotion is	Prepare new lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to instructions
	Not terminated before reading	Stop solution should be added to each well

UpingBio technology Co.,Ltd

If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the strips used and unused reagents, and then contact our company's technical support to solve the problem for you.

【statement】

1. Due to existing conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.

2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.

3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the

use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.

4. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.

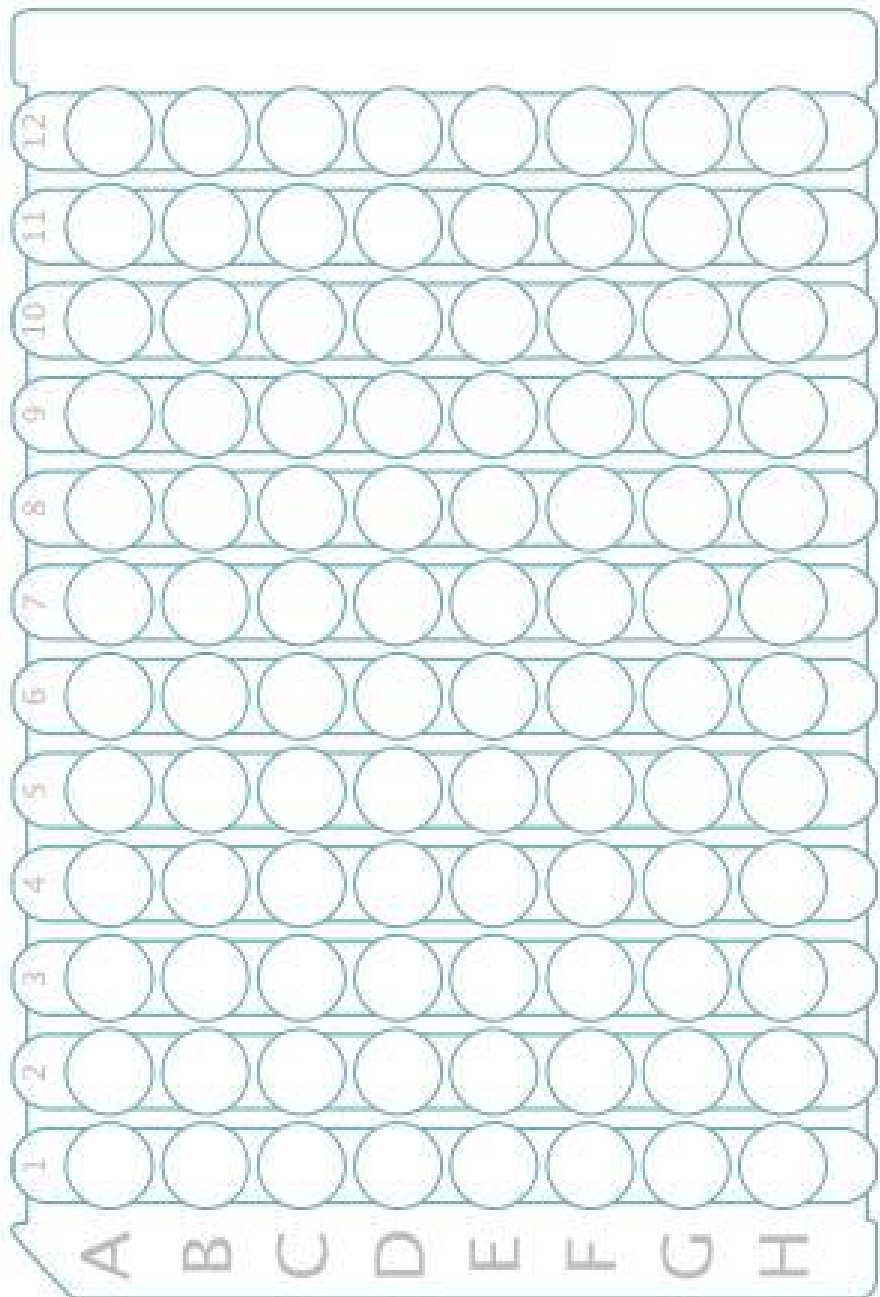
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may result. Please read the instructions carefully and adjust the instrument before the experiment.

6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.

8. This kit has not been compared with similar kits from other manufacturers or products using different methods to detect the same target, so inconsistent test results cannot be ruled out.

9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.



【Experimental experience】

【Experimental experience】