优品生物
UpingBio

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

Human Neprilysin (NEP) Quantitative Detection Kit (ELISA) Instructions for Use Specification: 96T/48T Catalog Number: SYP-H0222

Purpose: Used to detect human neprilysin (NEP) in serum, plasma, cell culture supernatant and other samples

concentration.

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 0.078 ng/ml.

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

Sensitivity: This kit recognizes natural human neprilysin (NEP) and has no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.

Detection range: 0.312 ng/ml – 10 ng/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 neprilysin (NEP) antibody (solid-phase antibody), add human neprilysin (NEP) calibrator and sample to be tested, and then add biotin-labeled antibody. After incubation and sufficient washing, HRP-coupled avidin is added. After incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen-biotin-labeled antibody is formed on the solid surface of the microplate. -Sandwich complex of avidinase. 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 enkephalin in the sample to be tested. The concentration of enzyme (NEP) is positively related. By fitting the calibrator curve, the concentration of human neprilysin (NEP) 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℃. 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℃.

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.

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.

【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. Make 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. Open the sealed bag after balancing to room temperature 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±10nm.

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

12、请勿使用其他批号或其他来源的试剂混合或替代本试剂盒中的试剂。

13、试验中所用的 EP 管和吸头均为一次性使用，严禁混用。

14、请勿使用过期的试剂。

【样品的准备和保存】

以下只是列出样品采集和保存的一般指南。所有样本采集保存过程中，不得使用叠氮钠做为防腐剂。样品如果不立即分析，应分装后冷冻保存，且避免反复冻融。

细胞培养上清：离心去除沉淀，立即分析或分装后-20℃冷冻保存。

血清：用干净试管收集血液，室温凝固 30 分钟，离心 2000×g 20 分钟，收集血清。立即分析或分装后-20℃冷冻保存。

血浆：采用肝素、柠檬酸盐或 EDTA 抗凝，抽血后 30 分钟内在 2-8℃离心 2000×g 20 分钟。为消除血小板的影响，建议在 2-8℃进一 步离心 10000×g 10 分钟。立即分析或分装后-20℃冷冻保存。

细胞裂解液：对于贴壁细胞，去除培养液，用 PBS、生理盐水或无血

清培养液洗一遍。加入适量裂解液，用移液器吹打数下，使裂解液和细胞充分接触。通常 10 秒后，细胞就会被裂解。对于悬浮细胞，离心收集细胞，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用移液器吹打把细胞吹散，用手指轻弹以充分裂解细胞。充分裂解后，10000—14000×g 离心 3-5 分钟， 取上清。立即分析或分装后-20℃冷冻保存。

尿液：用无菌管收集，离心 2000×g 20 分钟。仔细收集上清。如有 沉淀形成，应再次离心。

【试剂准备】

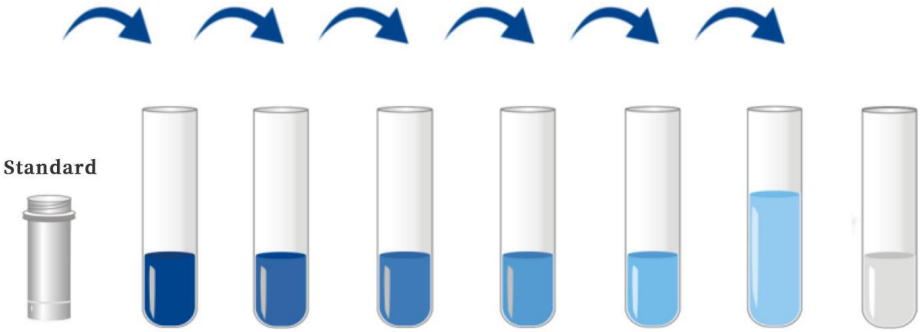
1、使用前，所有的组分都要至少复温 120min，确保充分复温到室温。

2、浓缩洗涤液：从冰箱取出的浓缩洗涤液，会有结晶产生，这属于正常现象，水浴加热使结晶完全溶解。浓缩洗涤液与蒸馏水，按 1:20 稀释，即 1 份的浓缩洗涤液，添加 19 份的蒸馏水。

【校准品稀释方法】

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 concentration of the mother solution of the calibrator after redissolution is 20 ng/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 μL calibrator ＆ sample diluent, pipet 500 μL from the 20 ng/ml calibrator mother solution into the first EP tube, mix well to prepare a 10 ng/ml calibrator working solution, and follow this step in sequence. Pipette and mix. As shown on the following page.



20ng/ml

Recommended dilution concentration of calibrator: It is recommended to prepare the following concentrations: 10, 5, 2.5, 1.25, 0.625,

0.312, 0 ng/ml, and used as the calibrator concentration value of the fitted 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 μL of calibrator of different concentrations to each of the calibrator holes. Add 50 μL of sample diluent to the sample diluent hole. Do not add it to the blank hole. Add the sample hole to be tested. Sample 50μL. 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.

1 minute, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If using an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

5. Except for the blank wells, add 100uL of HRP-labeled avidin to each well, cover the reaction plate with a sealing film, and incubate in a 37℃ water bath or incubator in the dark for 20 minutes.

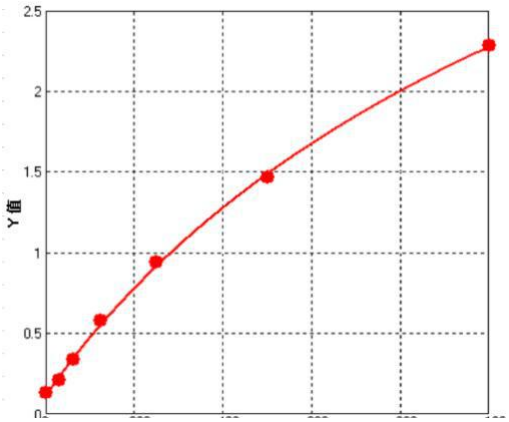
6. Repeat step 4.

7. Add 100μL of TMB chromogenic solution to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

8. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450 nm wavelength microplate reader.

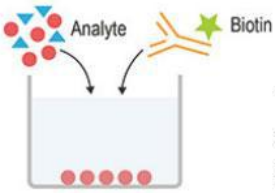
【Result calculation】

1. Use the concentration of the calibrator as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software] 2. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.

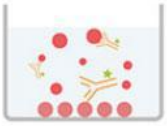


(Schematic diagram, for reference only)

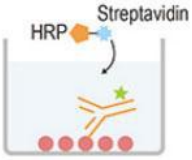
[Operation Summary]



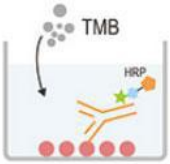
1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液，37℃孵育60分钟。



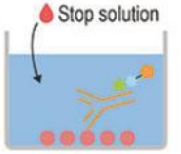
2、弃掉板内液体，洗板5次。



3、每孔加入100uL HRP酶结合物工作液37℃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℃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。

【problem analysis】

Problem Description	Possible Causes	Corresponding
Negative and positive control results are unstable	Incorrect liquid	Check pipettes and tips
	Equilibration time is too	Ensure sufficient
	Incomplete washing	Ensure the washing time and number of washes
Very weak or colorless	Incubation time too short	Ensure adequate
	Experimental	Use recommended
	Insufficient reagent	Check the liquid aspiration and addition
	Incorrect dilution	
	Enzyme label inactivation or substrate	Mix enzyme conjugate and substrate and check
Reading value is low	Microplate reader settings are incorrect	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

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. Limited by the existing conditions and scientific and technological level, it is not possible to conduct a 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.

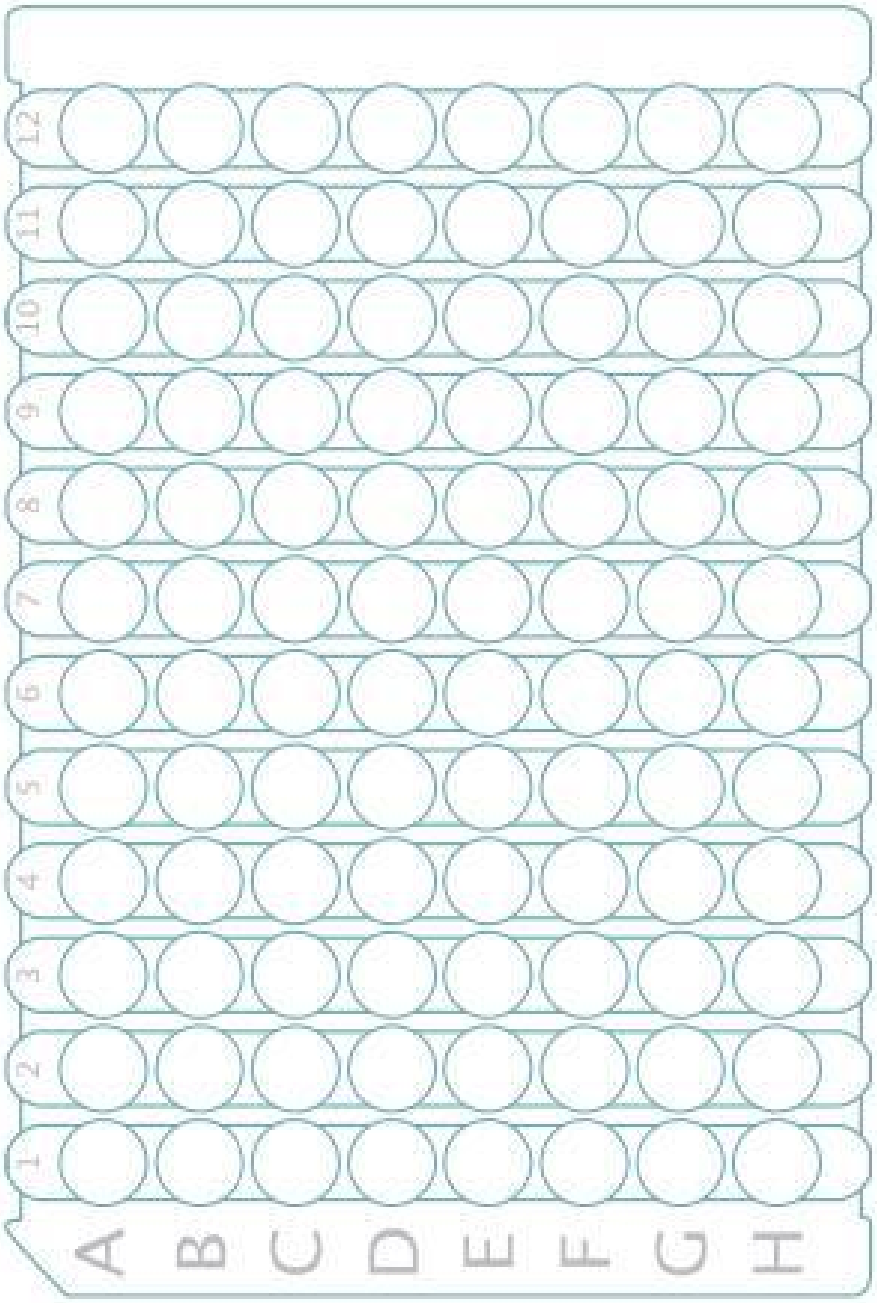
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may occur. Please read the instructions carefully and adjust the instrument before the experiment.

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 kit 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.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【Experimental experience】

【Experimental experience】