(仅供科研使用，不得用于临床诊断!)

人神经型一氧化氮合酶(NOS1/nNOS)

定量检测试剂盒（ELISA）使用说明书

规格：96T/48T 货号：SYP-H0207

用途：用于检测血清、血浆、细胞培养上清液等样本中人神经型一氧化氮合酶(NOS1/nNOS)的浓度。

使用前请仔细阅读说明书。如果有任何问题，请通过以下方式联系我们：

官方热线：400-999-8863

技术电话：18358180525

邮箱：UpingBio@163.com

公司网址：www.upingbio.com 具体保质期请见试剂盒外包装标签。请在保质期内使用试剂盒。联系时请提供产品货号、生产日期（见盒签），以便我们更高效 为您服务。

【试剂盒性能】

物理性能：各液体组分澄清透明、无沉淀或者絮状物。微孔板铝箔袋应真空包装，无破损漏气。

校准曲线线性：校准品剂量反应曲线相关系数 r 值，大于等于 0.9900。精密度：批内变异系数 CV%小于 10%；批间变异系数 CV%小于 15%。灵敏度：最低检出剂量小于 0.078 ng/ml。

回收率：回收率在 85%-115%之间。

敏感性：本试剂盒识别天然人神经型一氧化氮合酶(NOS1/nNOS)，与结构类似物无交叉。

稳定性：2℃-8℃保存，有效期 6 个月。

检测范围：0.312 ng/ml – 10 ng/ml。

【实验原理】

本试剂盒采用双抗体夹心法酶联免疫吸附试验（ELISA）。在预包被抗 人神经型一氧化氮合酶(NOS1/nNOS)抗体（固相抗体）的微孔酶标板中，加入人神经型一氧化氮合酶(NOS1/nNOS)校准品和待测样本，再加入生 物素标记抗体，经过温育与充分洗涤后，再加入 HRP 偶联的亲和素，经 过温育与充分洗涤，去除未结合的组分，在微孔板固相表面形成固相抗体-抗原-生物素标记抗体-亲和素酶的夹心复合物。加 TMB 显色液，产生蓝 色产物，在终止液作用下，最终转化为黄色，在酶标仪 450nm 波长上测 定吸光度（OD 值），吸光度（OD 值）与待测样品中人神经型一氧化氮合 酶(NOS1/nNOS)的浓度正相关。拟合校准品曲线，可以计算出样本中人 神经型一氧化氮合酶(NOS1/nNOS)的浓度。

【试剂盒组分与保存】

组分		数量	主要成分
校准品	High Standard	2 vial	校准品冻干粉
校准品复溶液	Reconstitution	2 vial	PBS
校准品＆样本稀释液	Standard & Sample Diluent	25mL	PBSTN
包被微孔板	Microelisa Stripplate	96T/48T	预包被固相抗体
生物素抗体	Bio-Antibody	10mL	生物素抗体
HRP 标记亲和素	HRP-Conjugate	10mL	HRP 标记亲和素
TMB 显色液	TMB	10mL	TMB
终止液	Stop Solution	6mL	酸性溶液
20×浓缩洗涤液	20X Wash Solution	25mL	0.05%Tween20
说明书	说明书	1 份	--
自封袋	自封袋	1 个	--
不干胶	不干胶	4 片	--

注意：1、使用前请检查试剂盒中试剂的标签和数量与表格是否一致。

2、试剂盒 2-8℃保存，不得使用过期试剂盒。

3、包被微孔板单次未使用完，要谨记密封放到 2-8℃保存。

4、复溶后的校准品仅限当天使用。

5、如果试剂盒的组份需要再次使用，请确保上一次使用之后没有被污染。

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. 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 shall 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×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 × 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.

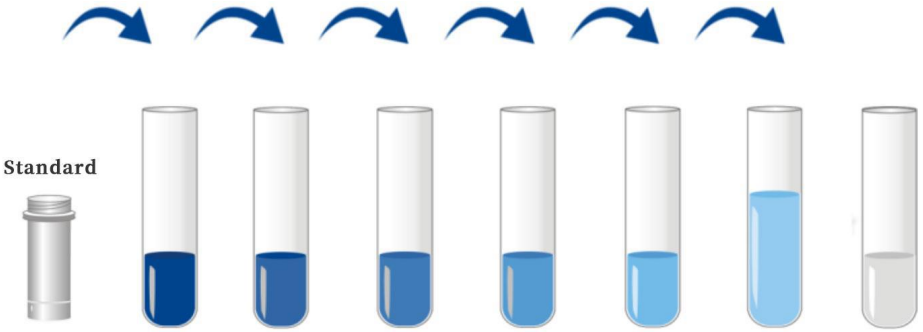
2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating

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.

[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 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 any to the blank hole. Add the test well to the sample hole. 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 you use 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.

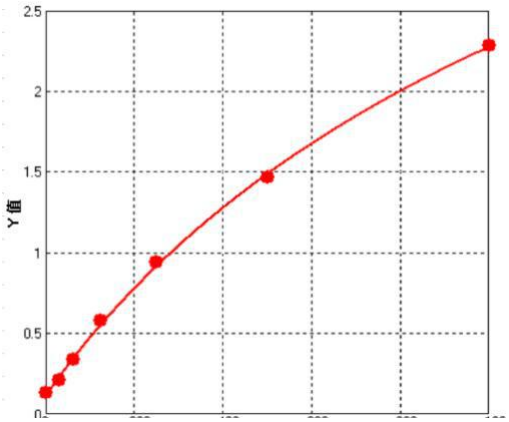
6. Repeat step 4.

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.

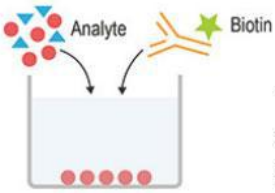
【Result calculation】

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.

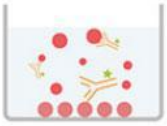


(Schematic diagram, for reference only)

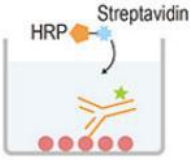
[Operation Summary]



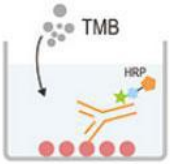
1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液，37℃孵育60分钟。



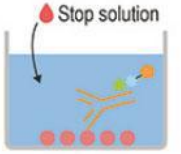
2、弃掉板内液体，洗板5次。



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. 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.

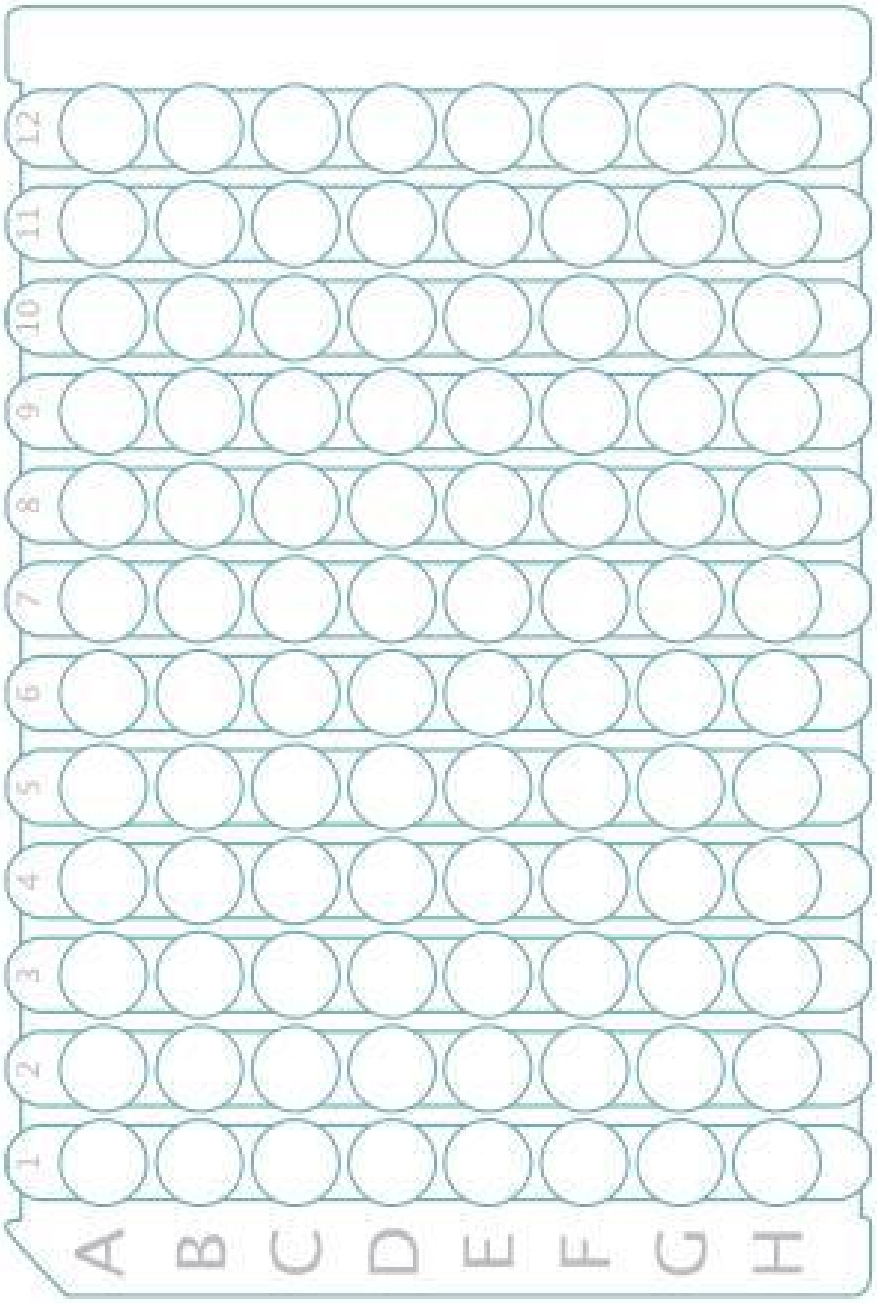
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may occur. Please read the instructions carefully and adjust the instrument before the experiment.

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 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.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【Experimental experience】

【Experimental experience】