(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Nitric Oxide (NO) Assay Kit

Catalog No.: BC00011

Size: 500T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086- 27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Nitric Oxide (NO) Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, Plasma, Urine , Tissue, Supernatant of cell lysate, etc.
Assay Type	Quantitative
Detection Instrument	Microplate reader (520-560 nm, optimal detection wavelength 540 nm)

Product Introduction

Nitric Oxide (NO) is widely distributed in the body, including the nervous, circulatory, respiratory, digestive, urogenital and other systems, especially in the nervous tissue. As an information substance between cells and within cells, it plays a role in signal transmission. It is a new type of biological messenger molecule and plays an important role in the physiological and pathological processes of the body.

Product Features

● This kit uses the classic Griess Reagent and optimizes its assay solution system to achieve a detection limit of 1μM and a perfect linear relationship in the range of 1-100μM.
● The detection speed is extremely fast, and it only takes 3 minutes to complete the determination of a standard curve or 5-10 samples.
● The sample range is wide and can detect the content of nitric oxide in cells or tissues and their culture medium. Phenol red and 10% serum have no obvious interference with the determination. The content of nitric oxide in serum, plasma and urine can also be detected.

Principle

Nitric oxide (NO) is an extremely unstable biological free radical that can be rapidly oxidized in vivo or in aqueous solution to form nitrite (NO_2^-) and nitrate (NO_3^-). Under acidic conditions, nitrite first reacts with p-aminobenzenesulfonic acid (sulfanilamide) to form a diazonium salt, and then reacts with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to form a purple-red azo compound. The concentration of the generated azo compound has a linear relationship with the concentration of NO, and the concentration of NO can be indirectly calculated by colorimetry.

Components

Serial number	Components	Size (500T)	Storage
Reagent 1	1M $NaNO_2$ Standard	1 ml	Store at -20℃, away from light.
Reagent 2	Griess Reagent I	25ml	Store at -20℃, away from light.
Reagent 3	Griess Reagent II	25ml	Store at -20℃, away from light.
Consumable 1	Microplate(96 wells)	5 plates	RT
Consumable 2	Plate Sealer	10 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months.
After opening the bottle, store at -20°C away from light, valid for one year; store at 4°C away from light, valid for half a year.

Experimental Preparation

- Sample processing

1. The sample is the supernatant of the culture medium and can be sampled directly. If there is precipitable matter, it is necessary to take the supernatant after centrifugation.
2. If the sample is a cell or tissue, it can be quickly frozen and thawed, then centrifuged and the supernatant can be taken. If the volume is less than 50μl , it can be diluted with redistilled water or 0.9% NaCl (correspondingly, the standard also needs to be diluted with redistilled water or 0.9% NaCl) .
3. Cells or tissues can also be lysed using Western or IP lysis buffer (without adding inhibitors) , and the standards should also be diluted accordingly. RIPA lysis buffer is not recommended.

- Preparation of the assay kit

1. Take out Griess Reagent I and II and return them to room temperature.
2. Dilute the standard (0-100 μM) with the solution used for the samples to be tested . For example, if the sample is cell culture supernatant and the cell culture medium is DMEM + 10% FBS , then the standard is diluted with DMEM + 10% FBS . Usually, the concentration of the standard can be prepared by half dilution method: 100 , 50, 25, 12.5, 6.25, 3.12, 1.56, 0μM .
Example: To prepare 120μl of a 100μM standard, take 60μl and add 60μl of diluent to prepare 120μl of a 50μM standard, and so on. The 0μM standard is the pure diluent.

Operation process

1. Add standards and samples to a 96-well plate at 50 μl/well .
2. Add 50 μl/ well of room temperature Griess Reagent I to each well .
3. Add room temperature Griess Reagent II to each well at 50 μl/ well .
4. Measure the absorbance at 540nm using an microplate reader.

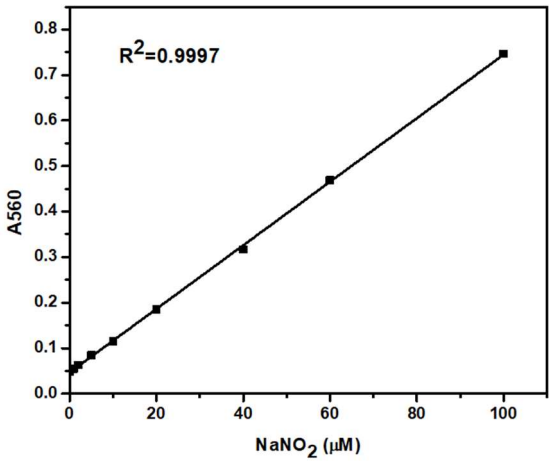
The operation table is as follows (for reference):

	Standard hole	Determination hole
Standards of different concentrations (μL)	50	--
Sample to be tested (μL)	--	50
Griess Reagent I (μL)	50	50
Griess Reagent II (μL)	50	50
The OD value of each well was measured by microplate reader at 540 nm.		

Note: If there is no 540nm filter, a 520-560nm filter can also be used.

Result calculation

The concentration of nitric oxide in the sample is calculated based on the standard curve. See the figure below for an example of a standard curve (showing the A 560 readings of different concentrations of $NaNO_2$ standards detected by this kit) for reference. In actual measurements, due to factors such as reaction conditions and different kit batches, the test results may differ from the example data.



Notes

1. This product is harmful to the human body. Please be careful when handling it and

take effective protection to avoid direct contact with the human body or inhalation.
2. If improper storage causes the solution to change color or precipitate, it means that the solution has expired. Please purchase a new kit.
3. It is not recommended to use RIPA lysis buffer to lyse cells or tissues. Using RIPA lysis buffer may produce precipitation in subsequent reactions, affecting the test.
4. For the determination of NO content in serum samples, for rough calculation, the standard can be directly diluted with water to calculate the NO concentration in the serum sample. For more accurate calculation, if the normal serum to be measured is common serum, the NO concentration in it can be found in the literature, and then the standard can be diluted with the serum with known NO concentration, so that a more accurate NO concentration can be obtained. Alternatively, the purpose can be achieved by diluting the standard with human or other animal serum of known concentration. Alternatively, the NO concentration in serum can be determined by referring to similar literature.
5. This product is for Research Use Only, and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.