FineTest®

Total Nitric Oxide Assay Kit

Catalog No.: K051

Size:50T/200T

Storage: -20°C for one year. NADPH, Nitrate Reductase, NaNO2 (1M), Griess Reagent I and Griess Reagent II should be stored away from light.

Kit components:

Item	50T	200T
Sample Diluent	15 mL	15 mL
NADPH	5 mg	5 mg
FAD	550μl	1.1 mL*2
Nitrate Reductase	250μl	1 mL
LDH Buffer	550μl	1.1 mL*2
LDH	500μl	1 mL*2
$NaNO_2$ (1M)	1 mL	1 mL
Griess Reagent I	3 mL	12 mL
Griess Reagent II	3 mL	12 mL
Product Description	1copy	1copy

Introduction

Total Nitric Oxide Assay Kit (Total NO Assay Kit) uses Nitrate reductase to reduce Nitrate to nitrite, and then uses the classic Griess Reagent for nitrite detection to determine total nitric oxide. Nitric oxide itself is extremely unstable and quickly metabolized into Nitrate and Nitrite in the cell. By detecting the total amount of Nitrate and Nitrite in the above method, the total amount of nitric oxide can be calculated.

This kit uses NADPH dependent Nitrate reductase. High levels of NADPH can interfere with subsequent detection, so The kit uses the method of LDH to remove NADPH, which makes the result more accurate.

The lower limit of nitrite detection is 2 μmol/l, and there is a good linear relationship in the range of 2-80 μmol/l. Samples with high concentrations can be diluted before testing.

A wide range of samples can be used to detect the nitric oxide content, including cell lysates, tissue lysates, cell or tissue culture fluids, serum, plasma or urine, etc. Phenol red and 10% serum did not interfere with the determination.

Depending on the concentration of nitric oxide in the sample, only 0-60 μL samples are required. The detection speed is fast, it only takes about 80 minutes to complete the detection.

Precautions for Use

The detection reaction must be carried out away from light.

RPMI 1640 and other culture medium containing high concentration of nitrate may easily interfere with the detection of this kit, so please try to avoid it. When RPMI 1640 culture medium is necessary, it must be replaced with other appropriate culture medium such as DMEM, MEM, F12, etc., or HBSS or PBS, etc., before nitric oxide detection.

Due to the reduction reaction in the detection process, any oxidation or reduction reagent that affects the reduction reaction should be avoided, such as the commonly used reducing agent DTT and mercaptoethanol.

This product is only limited to scientific research personnel, can not be used for clinical diagnosis or treatment, food or medicine, must not be stored in the ordinary residential.

For your safety and health, please wear clothes and wear disposable gloves.

Assay Procedure

1. Sample preparation

Samples containing high concentrations of protein such as serum or cell culture fluid containing high concentrations of serum may precipitate after the addition of Griess Reagent I. 50 μ l of sample can be used for testing by adding 50 μ l Griess Reagent I for precipitation. If precipitation occurs, the sample can be heated in a boiling water bath for 5 min to denaturate the protein and centrifuged 12,000g for 5 min to remove the supernatant for subsequent determination.

Do not use heparin anticoagulant plasma. Heparin anticoagulant plasma can precipitate when used with Griess Reagent.

For cell or tissue samples, a lysis buffer can be used for lysis. Urine samples usually need to be diluted 10-50 times with water before testing.

2. Standard dilution

The 1M NaNO2 was diluted to 2, 5, 10, 20, 40, 60, 80 μ mol/l with the solution used for sample preparation or dilution. If the sample is serum, the standard can be diluted with the sample diluent supplied with this kit or simply with appropriate solutions such as PBS or normal saline. Diluted standard products should be prepared and used now, not used after frozen storage.

3. Reagent preparation

A. Add about 1mL of double distilled water to 5mg NADPH, mix it upside down, dissolve it, and then add double distilled water to 3mL to prepare 2mM NADPH. Except for the part immediately used, the rest of the NADPH solution must be immediately packaged and frozen at -70℃.

B. FAD has been formulated in an appropriate solution and can be properly separated and stored at -20℃ or -70℃.

C. Nitrate Reductase and LDH were taken out before use and placed in an ice bath for use (note that storage at -20℃ immediately after use), and other reagents in the kit were stored in an ice bath after dissolution. Griess Reagent I and Griess Reagent II should be at room temperature before use.

4. Refer to the following table to add standard, samples and reagents in turn and conduct

corresponding tests:

	Negtive control	standard	samples
standard	-	60 μ l	-
samples	-	-	x μ l
Sample diluent	60 μ l	-	(60-x) μ l
NADPH(2mM)	5 μ l	5 μ l	5 μ l
FAD	10 μ l	10 μ l	10 μ l
Nitrate Reductase	5 μ l	5 μ l	5 μ l
After mixing, incubate at 37℃ for 30min			
LDH buffer	10 μ l	10 μ l	10 μ l
LDH	10 μ l	10 μ l	10 μ l
After mixing, incubate at 37℃ for 30min			
Griess Reagent I	50 μ l	10 μ l	10 μ l
Griess Reagent II	50 μ l	10 μ l	10 μ l
After mixing, A540 was determined after incubation at room temperature (20-30℃) for 10 min			

NOTE:

（1） The reaction must be carried out in the dark.If a 96-well plate is used for detection, wrap the 96-well plate with aluminum foil to protect it from light.

（2） Upper limit for sample is 60 μ l. 2-3 Wells added with 200 μ L of water or PBS can be set as negative control at the same time, and only water or PBS can be added to these 2-3 Wells without any other reagents.

（3） Add the Griess Reagent I and mix gently. After each mixing, centrifuge for a few seconds at 1000-3000g to precipitate the liquid to the bottom of the tube. At the same time, avoid bubbles in the detection holes, so as not to interfere with the detection results.

（4） When detecting, if no 540nm filter is available, 520-560nm filter can also be used. If no microplate reader or suitable filter is available, quantitative analysis can also be carried out in appropriate graphics software after taking photos by digital camera. Photographic colorimetric standards require a finer concentration gradient.

5. Calculate the concentration of nitric oxide in the sample according to the standard curve.