Technical Manual

Nitric Oxide (NO) Colorimetric Assay Kit

- Catalogue Code: MAES0051
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.97-700 µmol/L

Sensitivity:

0.97 µmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

NO is a kind of highly reactive free radical, which has the function of the second messenger and neurotransmitter, and it is also a kind of effector molecule, which has a wide range of physiological functions in vivo, such as relax vascular smooth muscle, regulate cerebral blood flow, mediate cytotoxic effect and immune regulation, participate in learning and memory, etc. Half life of NO is very short. NO in blood is mainly produced by vascular endothelial cells, vascular smooth muscle cells, platelets, macrophages and so on. It exists in the form of nitrate and nitrite, and the concentration of NO can calculate indirectly by the concentration of nitrate and nitrite.

3. Intended Use

This kit can be used for detection of nitric oxide (NO) in serum, plasma, animal and plant tissue samples.

4. Detection Principle

NO is easily oxidized to form NO_2^- in vivo or in aqueous solution, and a reddish azo compound is formed with the color developing agent, and the concentration of the azo compound is linearly related to the concentration of NO. The concentration of NO can be calculated indirectly by measuring the OD value at 550 nm.

5. Kit components & storage

Item	Specification	Storage
Sulphate Solution	50 mL × 4 vials	2-8°C, 6 months
Alkali Reagent	50 mL × 2 vials	2-8°C, 6 months
Chromogenic Agent A	38 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent B	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Acid Solution	25 mL × 1 vial	2-8°C, 6 months
Sodium Nitrite Standard	Lyophilized × 2 vials	-20°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (550 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

The supernatant for assay should not contain sediment, otherwise it will affect the results.

7. Reagent preparation:

1. Bring all reagent to room temperature before use.

2. Preparation of chromogenic agent B working solution: Dissolve a vial of chromogenic agent B with 37.5 mL of double distilled water fully. The prepared solution can be stored at 4°C for 2 months with avoid direct sunlight.

3. Preparation of chromogenic reagent: Mix the chromogenic agent A, chromogenic agent B working solution and acid solution at a ratio of 3:3:2 fully. Prepare the fresh solution before use and it can't be used when its color gets darker.

4. Preparation of sodium nitrite standard (2 mmol/L): Dissolve standard lyophilized with 2 mL of double distilled water. Prepare the needed amount before use.
   Preparation of sodium nitrite standard solution (40 µmol/L): Dilute the standard solution (2 mmol/L) with distilled water at a ratio of 1:49 and mix fully. Prepare the fresh solution before use.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.97-700 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
Human serum	1
Human plasma	1
10% Mouse liver tissue homogenization	1
Rat serum	1
Rat plasma	1
10% Epipremnum aureum tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 550 nm

10. Operation Steps

1. **Blank tube:** Take a* mL of double distilled water to 1.5 mL EP tubes.
   Standard tube: Take a* mL of sodium nitrite standard solution (40 μmol/L) to 1.5 mL EP tubes.
   Sample tube: Take a* mL of sample to 1.5 mL EP tubes.
   Note: a* = Sample volume= Standard volume. For serum or plasma samples, a* is 0.2-0.3 mL. For tissue, a* is 0.1-0.3 mL.

2. Add 1.6 mL of sulphate solution and mix fully with a vortex mixer.

3. Add 0.8 mL of alkali reagent and mix fully with a vortex mixer.

4. Stand for 15 min at room temperature, centrifuge at 3100 g for 10 min. (If there is precipitate in the supernatant, please transfer the supernatant to a new EP tube and centrifuge again.)

5. Take 1.6 mL of supernatant to the corresponding tubes for chromogenic reaction.

6. Add 0.8 mL of chromogenic reagent to each tube, oscillate for 2 min and stand at room temperature for 20 min.

7. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 550 nm with 1 cm optical path cuvette.

Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	a*		
40 μmol/L sodium nitrite standard solution (mL)		a*	
Sample (mL)			a*
Sulphate solution (mL)	1.6	1.6	1.6
Alkali reagent (mL)	0.8	0.8	0.8
Mix fully and stand for 15 min, centrifuge at 3100 g for 10 min, take the supernatant for chromogenic reaction.			
Supernatant (mL)	1.6	1.6	1.6
Chromogenic reagent (mL)	0.8	0.8	0.8
Mix fully and stand at room temperature for 20 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 550 nm with 1 cm optical path cuvette.			

11. Calculations

1. Serum (plasma) sample:

$$\text{NO content (μmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

$$\text{NO content (μmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

ΔA1: $OD_{Sample} - OD_{Blank}$
ΔA1: $OD_{Standard} - OD_{Blank}$
c: Concentration of sodium nitrite, 40 μmol/L.
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	0.97-700 μmol/L
Sensitivity	0.97 μmol/L
Average recovery rate (%)	99
Average inter-assay CV (%)	5.2
Average intra-assay CV (%)	3.4

Analysis

Take 0.3 mL of 10% mouse liver tissue homogenate, carry the assay according to the operation table.

The results are as follows:
The average OD value of the sample is 0.010, the average OD value of the blank is 0.004, the average OD value of the standard is 0.065, the concentration of protein in sample is 8.65 gprot/L, and the calculation result is:

$$\textbf{NO content (μmol/gprot)} = \frac{\textbf{0.010-0.004}}{\textbf{0.065-0.004}} \times \textbf{40} \div \textbf{8.65} = \textbf{0.45 μmol/gprot}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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