



PRODUCT INFORMATION & MANUAL

Nitrite Assay Kit (Colorimetric) *NBP3-25890*

For research use only.
Not for diagnostic or therapeutic
procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Nitrite Assay Kit (Colorimetric)

Catalog No: NBP3-25890

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 1.36 $\mu\text{mol/L}$

Detection range: 1.36-500 $\mu\text{mol/L}$

Average intra-assay CV (%): 2.6

Average inter-assay CV (%): 3.3

Average recovery rate (%): 97

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

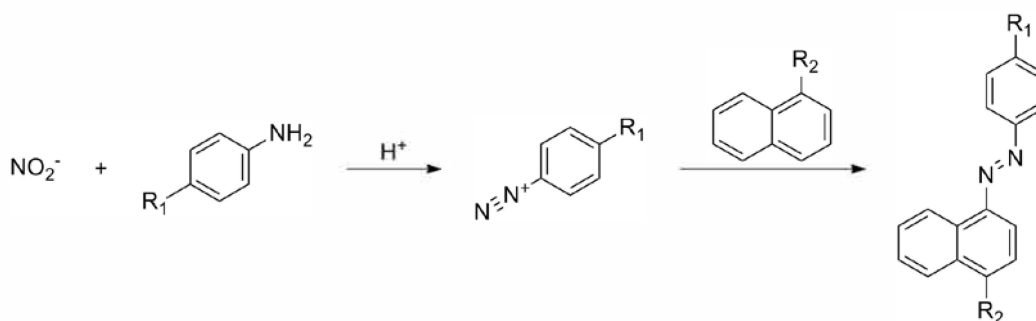
This kit can be used to measure the nitrite (NO_2^-) content in serum, plasma, saliva, tissue, cells, culture supernatant samples.

▲ Background

Nitrite and nitrate are important chemical components in almost all living organisms. In vivo, nitrite and nitrate come from the NO oxidation pathway without excessive uptake of nitrite and nitrate.

▲ Detection principle

Nitrite can react with chromogenic agent producing light red azo-compound. The content of nitrite can be calculated indirectly by measuring the OD value at 550 nm.



▲ **Kit components & storage**

Item	Component	Specification	Storage
Reagent 1	Saline Solution	50 mL × 2 vials	2-8°C , 12 months
Reagent 2	Alkali Reagent	50 mL × 1 vial	2-8°C , 12 months
Reagent 3	Chromogenic Agent A	Powder ×1 vial	2-8°C , 12 months, shading light
Reagent 4	Chromogenic Agent B	Powder × 1 vial	2-8°C , 12 months, shading light
Reagent 5	Acid Solution	12 mL × 1 vial	2-8°C , 12 months
Reagent 6	Sodium Nitrite Standard	Powder × 1 vial	-20°C , 12 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲ **Materials prepared by users**

 Instruments

Spectrophotometer (550 nm), Micropipettor, Vortex mixer, Centrifuge

 Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲ Precautions

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

▲ The key point of the assay

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

Pre-assay preparation

▲ Reagent preparation

1. Preparation of reagent 3 working solution

Dissolve a vial of reagent 3 powder with 30 mL double distilled water (60-70°C) and mix fully. it can be store at 2-8°C with shading light for 3 months.

2. Preparation of reagent 4 working solution

Dissolve a vial of reagent 4 powder with 12 mL double distilled water and mix fully. The prepared solution can be store at 2-8°C with shading light for 2 months. If the reagents appear darkened color, it should be abandon.

3. Preparation of chromogenic agent

Mix reagent 3 working solution, reagent 4 working solution and reagent 5 at a ratio of 2.5: 1: 1. Prepared the fresh solution before use and store at 2-8°C with shading light. It can't be use when the color is darkened.

4. Preparation of 2 mmol/L sodium nitrite standard

Dissolve reagent 6 with 2 mL of double distilled water. Prepare the needed amount before use.

5. Preparation of 100 µmol/L sodium nitrite standard

Mix 2 mmol/L sodium nitrite standard with distilled water at a ratio of 1:19 fully. Prepare fresh solution before use.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

1. DTT, mercaptoethanol, ascorbic acid and other reducing substances should not be added in the samples.
2. The kit can't be used for the detection of cell supernatant containing phenol red or other colored substance.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.36-500 $\mu\text{mol/L}$).

Assay protocol

▲ Detailed operation steps

- (1) **Blank tube:** Take a^* mL of double distilled water to 2 mL EP tubes.
Standard tube: Take a^* mL of 100 $\mu\text{mol/L}$ sodium nitrite standard solution to 2 mL EP tubes.
Sample tube: Take a^* mL of sample to 2 mL EP tubes.
[Note]: a^* = Volume of sample = Volume of standard = Volume of double distilled water. For serum or plasma samples, a^* is 0.2-0.4 mL. For tissue or cell homogenates, a^* is 0.1-0.2 mL.
- (2) Add 0.8 mL of reagent 1 and mix fully with a vortex mixer.
- (3) Add 0.4 mL of reagent 2 and mix fully with a vortex mixer.
- (4) Stand for 10 min at room temperature, centrifuge at 2000 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 0.8 mL of supernatant to the corresponding tubes for chromogenic reaction.
- (6) Add 0.4 mL of chromogenic reagent to each tube, mix fully and stand at room temperature for 15 min.
- (7) Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 550 nm with 0.5 cm optical path cuvette.

▲ Summary operation table

1. Pre-treatment

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	a*		
100 μmol/L sodium nitrite standard solution (mL)		a*	
Sample (mL)			a*
Reagent 1 (mL)	0.8	0.8	0.8
Reagent 2 (mL)	0.4	0.4	0.4
Mix fully and stand for 10 min, centrifuge at 2000 g for 10 min, take the supernatant for chromogenic reaction.			

2. Chromogenic reaction

	Blank tube	Standard tube	Sample tube
Supernatant (mL)	0.8	0.8	0.8
Chromogenic reagent (mL)	0.4	0.4	0.4
Mix fully and stand at room temperature for 15 min. Set the spectrophotometer to zero and measure the OD values of each tube.			

▲ Calculation

1. Serum (plasma) and other liquid sample:

$$\text{NO}_2^- (\mu\text{mol/L}) = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue and cells sample:

$$\text{NO}_2^- (\mu\text{mol/gprot}) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{\text{pr}}$$

Note:

ΔA_1 : $\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}$

ΔA_2 : $\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}$

c: Concentration of standard, 100 $\mu\text{mol/L}$.

f: Dilution factor of sample before test.

C_{pr} : Concentration of protein in sample, gprot/L.

Appendix I Data

▲ Example analysis

Take 0.3 mL of human serum sample, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.033, the average OD value of the blank is 0.003, the average OD value of the standard is 0.476, and the calculation result is:

$$\text{NO}_2^- \text{ content}(\mu\text{mol/L}) = (0.033 - 0.003) \div (0.476 - 0.003) \times 100 = 6.34 \text{ } (\mu\text{mol/L})$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge 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.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g 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.

▲ Cell culture supernatant

Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80°C for a month.

▲ 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 homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4°C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

▲ Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) = 1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 3100 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80 °C for a month.

▲ Saliva

Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4 °C. Take the supernatant and preserve it on ice for detection.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Hord N G, Tang Y, Bryan N S. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *American Journal of Clinical Nutrition*, 2009, 90(1): 1-10.
2. Sun J, Zhang X J, Broderick M, Fein H. Measurement of Nitric Oxide Production in Biological Systems by Using Griess Reaction Assay. *Sensors*, 2003, 3(8): 276-284.