Nitrite Colorimetric Assay Kit

Catalog No: E-BC-K070-S

Method: Colorimetric method

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

Instrument: Spectrophotometer

Sensitivity: 1.36 μmol/L

Detection range: 1.36-500 μmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

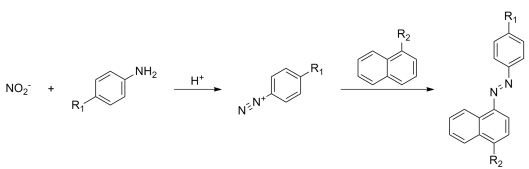
This kit can be used to measure the nitrite (NO_2^-) content in serum, plasma, saliva, tissue, cells, culture supernatant samples.

▲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℃ , 6 months
Reagent 2	Alkali Reagent	50 mL × 1 vial	2-8℃ , 6 months
Reagent 3	Chromogenic Agent A	Powder ×1 vial	2-8℃ , 6 months, shading light
Reagent 4	Chromogenic Agent B	Powder × 1 vial	2-8℃ , 6 months, shading light
Reagent 5	Acid Solution	12 mL × 1 vial	2-8℃ , 6 months
Reagent 6	Sodium Nitrite Standard	Powder × 1 vial	-20℃ , 6 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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

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
   Dissolve a vial of reagent 3 powder with 30 mL double distilled water (60-70℃) and mix fully. it can be store at 2-8℃ with shading light for 3 months.
2. Preparation of reagent 4
   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℃ with shading light for 2 months. If the reagents appear darkened color, it should be abandon.
3. Preparation of chromogenic agent
   Mix reagent 3, reagent 4 and reagent 5 at a ratio of 2.5: 1: 1. Prepared the fresh solution before use and store at 2-8℃ 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 μmol/L).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	550 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

(1) Blank tube: Take a* mL of double distilled water to 2 mL EP tubes.
Standard tube: Take a* mL of 100 μmol/L sodium nitrite standard solution to 2mL 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.

▲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 with double distilled water and measure the OD values of each tube at 550 nm with 0.5 cm optical path cuvette.			

▲Calculation

1. Serum (plasma) and other liquid sample:

$$NO_2^- (\mu mol/L) = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue and cells sample:

$$NO_2^- (\mu mol/gprot) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

Note:

ΔA: $OD_{Sample} - OD_{Blank}$

ΔA: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 100 μmol/L.

f: Dilution factor of sample before test.

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	1.36-500 μmol/L	Average intra-assay CV (%)	2.6
Sensitivity	1.36 μmol/L	Average inter-assay CV (%)	3.3
Average recovery rate (%)	97		

▲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:

NO_2^- content(μmol/L)=(0.033-0.003)÷(0.476-0.003)×100=6.34 (μmol/L)

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . 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℃ 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℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ 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 (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

▲ Saliva

Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4℃ . 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).

▲ Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.