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

Catalog No：E-BC-K070-S

Specification：50 assays(48 samples)/ 100 assays(96 samples)

Measuring instrument：Spectrophotometer（550 nm）

Detection range：1.36-500 μmol/L

Elabscience®Nitrite Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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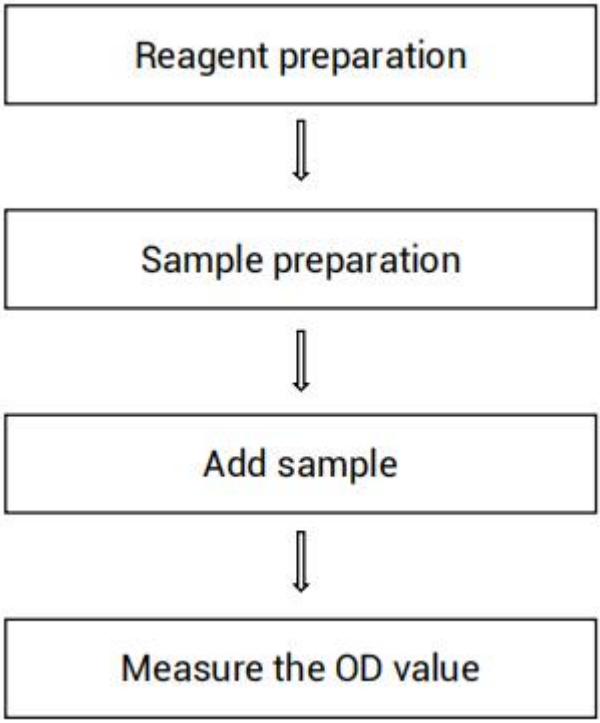
Assay summary



Intended use

This kit can be used to measure the nitrite (NO2-) content in serum, plasma, saliva, tissue, cells, culture supernatant samples.

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	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Saline Solution	50 mL ×1 vial	50 mL × 2 vials	2-8℃, 12 months
Reagent 2	Alkali Reagent	25 mL ×1 vial	50 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent A	Powder × 1 vial	Powder ×1 vial	2-8℃, 12 months shading light
Reagent 4	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 5	Acid Solution	6 mL ×1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 6	Sodium Nitrite Standard	Powder × 1 vial	Powder × 1 vial	-20℃, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Equilibrate reagents to room temperature before use.

② Preparation of chromogenic agent A working solution：
Dissolve one vial of chromogenic agent A with 30 mL of double distilled water (60-70℃), mix well to dissolve. Store at 2-8°C for 3 months protected from light.

③ Preparation of chromogenic agent B working solution：
Dissolve one vial of chromogenic agent B with 12 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 2 months protected from light. If the reagents appear darkened color, it should be abandon.

④ Preparation of chromogenic agent：
Before testing, please prepare sufficient mersuring working solution according to the test wells. For example, prepare 450 μL of chromogenic agent (add 250 μL of chromogenic agent A working solution, 200 μL of chromogenic agent B working solution and 200 μL of acid solution, mix well). The chromogenic agent should be prepared on spot. Store at 2-8°C for 2 days protected from light.

⑤ Preparation of 2 mmol/L sodium nitrite standard：
Dissolve one vial of Sodium Nitrite Standard with 2 mL of double distilled water, mix well to dissolve. The 2 mmol/L sodium nitrite standard solution should be prepared on spot.

⑥ Preparation of 100 μmol/L sodium nitrite standard：
Dilute 20 μL of 2 mmol/L sodium nitrite standard with 380 μL of double distilled water, mix well. The solution should be prepared on spot.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 30 mg tissue in 270 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

The key points of the assay

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

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 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 saline solution and mix fully with a vortex mixer.

③ Add 0.4 mL of alkali reagent 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.

Calculation

The sample:

1. Serum (plasma) sample:

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

2. Tissue and cells sample:

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

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$.

c: Concentration of standard, 100 μmol/L.

f: Dilution factor of sample before test.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	12.80	156.00	368.00
%CV	2.8	2.4	2.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	12.80	156.00	368.00
%CV	3.1	3.2	3.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	88.5	264	403
Observed Conc. (μmol/L)	87.6	256.1	382.9
recovery rate(%)	99	97	95

Sensitivity

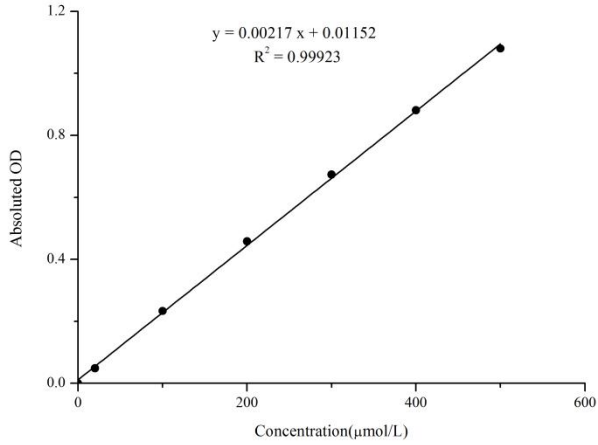
The analytical sensitivity of the assay is 1.36 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	20	60	100	200	300	400	500
Average OD	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Absoluted OD	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.051



Appendix Π Example Analysis

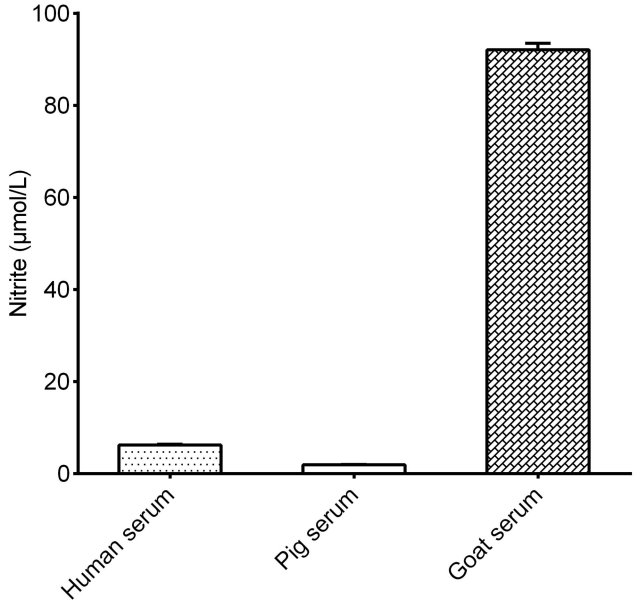
Example analysis：

For human serum, take 5 μL of human serum and carry the assay according to the operation steps. 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:

$$\underset{(\mu mol/L)}{NO_2^- \text{ content}} = \frac{(0.033-0.003)}{(0.476-0.003)} \times 100 = 6.34\ \mu mol/L$$

Detect human serum (A*=0.3 mL), pig serum (A*=0.2 mL), goat serum (A*=0.2 mL) according to the protocol, the result is as follows：



Appendix III Publications

1. Mosalam E M , Elberri A I , Abdallah M S ,et al.Mechanistic Insights of Neuroprotective Efficacy of Verapamil-Loaded Carbon Quantum Dots against LPS-Induced Neurotoxicity in Rats[J].International Journal of Molecular Sciences, 2024, 25(14).DOI:10.3390/ijms25147790.
2. Satyam S M , Bairy L K , Rehman A ,et al.Unlocking Synergistic Hepatoprotection: Dapagliflozin and Silymarin Combination Therapy Modulates Nuclear Erythroid 2-Related Factor 2/Heme Oxygenase-1 Pathway in Carbon Tetrachloride-Induced Hepatotoxicity in Wistar Rats[J].Biology (2079-7737), 2024, 13(7).DOI:10.3390/biology13070473.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.