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Nitrite Assay Kit

Catalog No.: BC00040

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Nitrite Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, animal and plant tissues
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (550 nm)
Range	3.125-50μM
Sensitivity	1.609μM

Product Introduction

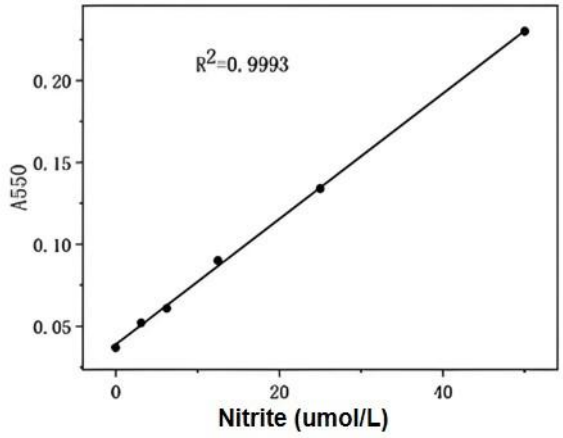
Nitrite is widely present in the human environment and is the most common nitrogen-containing compound in nature.

Detection principle

NO^{2-} reacts with the color developer to generate a light red azo compound. The concentration of the generated azo compound has a linear relationship with the concentration of NO^{2-}. The concentration of NO^{2-} can be determined by colorimetry . The role of reagents 1 and 2 is to remove the interference of colored substances in the sample. There is a maximum absorption peak at 550nm, and the absorbance value is proportional to the concentration within a certain range.

When this kit is used to test tissue samples, the total protein concentration needs to be measured. We recommend the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife.

The figure below shows the standard curve for the determination of nitrite by this kit. The following standard curve is for reference only:



Product composition

Serial Number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	Saline solution	50 mL×2 bottles	store at 2-8℃ after opening
Reagent 2	Alkaline solution	50 mL×1 bottle	store at 2-8℃ after opening
Reagent 3	Chromogen A	Powder × 1	store at 2-8℃ after opening
Reagent 4	Chromogen B	Powder × 1	store at 2-8℃ after opening
Reagent 5	Acid solution	12 mL×1 bottle	store at 2-8℃ after opening
Reagent VI	Sodium Nitrite	Powder × 1	-20℃
Consumables 1	96-well ELISA	1 plate	RT
Consumables 2	96-well	2 pieces	RT

Note: The reagents must be stored strictly according to the storage conditions in the table above. Reagents from different test kits cannot be mixed. For reagents with a small volume, please centrifuge before use to avoid insufficient amount of reagent.

Storage conditions

The unopened kit can be stored at -20℃ for 12 months. After opening, it can be stored at 4℃ for 6 months.

Preparation before the experiment

Sample processing

1. Liquid samples such as serum and plasma: can be measured directly.
2. Tissue samples: routine homogenization (homogenization medium is PBS (0.01 M, pH 7.4). After homogenization, centrifuge at 4°C, 10,000×g for 10 min, take the supernatant and place it on ice for testing. Reserve part of the supernatant for protein concentration determination.
3. Dilution of samples: Before formal testing, 2-3 samples with large expected differences should be selected and diluted into different concentrations for preliminary experiments. According to the results of the preliminary experiments, the appropriate dilution multiple should be selected.

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

· Preparation of the kit

1. The reagents in the kit were equilibrated to room temperature.
2. Preparation of reagent 3 working solution: Add reagent 3 powder to 30 mL of double distilled water at 60-70℃, stir to fully dissolve, and store at 2-8℃ away from light for 3 months.
3. Preparation of Reagent IV working solution: Add Reagent IV powder to 12 mL of double distilled water, stir to fully dissolve, and store at 2-8°C away from light for 2 months. If the reagent becomes darker in color, discard it.
4. Preparation of color developer: Mix reagent 3 working solution: reagent 4 working solution: reagent 5 in a volume ratio of 2.5:1:1, prepare before use, and store at 2-8℃ away from light for 2 days.
5. Preparation of 2 mmol/L standard: Dissolve 1 vial of reagent VI in 2 mL of double distilled water and use immediately after preparation.
6. Preparation of 100 μmol/L standard: Mix 2 mmol/L standard and double distilled water in a volume ratio of 1:19 and prepare immediately before use.
7. Dilution of standards of different concentrations: dilute the prepared 100 μmol/L standard with double distilled water in half to 50, 25, 12.5, 6.25, 3.125, and 0 (blank well) μmol/L.

Operation Process

Key points of the experiment

Add reagents 1 and 2, mix well, and the supernatant after centrifugation must be clarified. If it is turbid, centrifuge again.

1. Blank tube: Take A mL of double distilled water and add it to a 2 mL EP tube;
   Standard tube: Take A mL of 100 μmol/L sodium nitrite standard and add it to a 2 mL EP tube;
   Assay tube: Take A mL of the sample to be tested and add it to a 2 mL EP tube.
   (A is the sample volume = standard volume = double distilled water volume; serum (plasma) reference sampling volume: 0.2-0.4 mL, tissue homogenate reference sampling volume 0.1-0.2 mL)
2. Add 0.8 mL of reagent A to each tube in step "1" and vortex to mix.
3. Add 0.4 mL of reagent 2 to each tube in step "2" and vortex to mix.
4. Let stand at room temperature for 10 min, and centrifuge at 2000×g for 10 min. (If the supernatant contains some precipitate, transfer the supernatant to a new EP tube and centrifuge again)
5. Take 0.1 mL of the supernatant and add it to each well of the ELISA plate.
6. Add 0.05 mL of developer to each ELISA well in step "5" , mix well, let stand at room temperature for 15 min, and measure the OD value at a wavelength of 550 nm .

The operation table is as follows:

	Blank tube	Standard tube	Determination tube
Double distilled water (mL)	A	--	--
Sodium nitrite standards of different	--	A	--
Sample to be tested (mL)	--	--	A
Reagent 1 (mL)	0.8	0.8	0.8
Reagent 2 (mL)	0.4	0.4	0.4
Mix well, let stand at room temperature for 10 min, centrifuge at 2000Xg for 10 min, take the			
Supernatant (mL)	0.1	0.1	0.1
Color developer (mL)	0.05	0.05	0.05
The plate was vibrated for 5 seconds on the microplate reader, allowed to stand at room temperature for 15 minutes, and the OD value of each well was measured at a wavelength of 550 nm.			

Result Calculation

Standard fitting curve: y = ax + b

Serum (plasma) nitrite concentration calculation formula:

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

Tissue nitrite concentration calculation formula:

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

Annotation:

ΔA1: Sample OD value - Blank OD value

ΔA2: Standard OD value - Blank OD value

c: Standard concentration

f: dilution factor of the sample before adding it to the detection system

Cpr: protein concentration of the sample to be tested (gprot/L)

Question	Possible causes	Suggested Solutions

Large difference in duplicate holes	Not strictly following the instructions	Strictly follow the instructions
Very low color development in samples and standards	Incubation time is too short	Ensure adequate incubation time
Sample value not measured	Sample dilution number is too large	Select appropriate dilution factor and retest
	The sample has been stored for too long or Improper storage	Take a fresh sample and retest
Sample measurement result>500umol/L	Sample concentration is too high	Select appropriate dilution factor and retest
Low readings	Detection with inappropriate wavelength	Choose the right detection wavelength

Notes

1. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom and will not bear any legal liability.
2. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
3. Please wear lab coats and latex gloves for protection during the experiment.
4. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
5. If the sample being tested is not among the sample types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.
6. The final experimental results are closely related to the effectiveness of the reagents, the

relevant operations of the experimenter, the experimental environment and other factors. Our company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible usage of the sample before use and reserve sufficient samples.