PRODUCT INFORMATION & MANUAL

Nitric Oxide Assay Kit (Colorimetric)

NBP3-24527

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

Nitric Oxide (NO) Colorimetric Assay Kit (Nitrate Reductase Method)

Catalog No: NBP3-24527

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 1.38 μmol/L

Detection range: 1.38-40 μmol/L

Average intra-assay CV (%): 6

Average inter-assay CV (%): 8

Average recovery rate (%): 95

▲ 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

The kit can detect the concentration of NO in serum, plasma, urine, tissue, cell and supernatant of cell samples.

▲Detection principle

Nitric oxide (NO) has active chemical properties and is quickly metabolized into NO^{2-} and NO^{3-} in the body, while NO^{2-} is further transformed into NO^{3-}. This method uses nitrate reductase specifically to reduce NO^{3-} to NO^{2-}, and the concentration of NO is measured by the color depth.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Reagent	Powder × 4 vials	-20℃ , 12 months, shading light
Reagent 2	Substrate	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Sulfate Solution	3 mL × 1 vial	-20℃ , 12 months
Reagent 4	Alkaline Reagent	1.5 mL × 1 vial	-20℃ , 12 months
Reagent 5	Chromogenic Agent A	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 6	Chromogenic Agent B	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 7	Acid Reagent	3 mL × 1 vial	-20℃ , 12 months
Reagent 8	1 mmol/L Standard	1.5 mL × 2 vials	-20℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

centrifuge, Vortex mixer, 37℃ Incubator, Centrifuge, microplate reader (520-550 nm, optimum wavelength: 530 nm)

⚠ 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 points of the assay

1. The prepared chromogenic working solution should be stored with shading light.
2. After adding the enzyme working solution and sample or standard to the EP tube, it should be mixed fully.
3. Avoid the precipitation at the bottom of EP tube when take the supernatant of the incubation reaction.

Pre-assay preparation

▲ Reagent preparation

1. Preserve reagent 1 and reagent 2 on ice for detection, and bring other reagents to room temperature before use.
2. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 powder with 1 mL double distilled water and mix fully. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C with shading light for 6 h.
3. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 powder with 5 mL double distilled water and mix fully. The prepared solution can be divided into smaller packages and stored at -20°C with shading light for 3 days.
4. Preparation of enzyme working solution:
   Mix the reagent 1 working solution and reagent 2 working solution at a ratio of 1: 1. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C with shading light for 6 h.
5. Preparation of reagent 5 working solution:
   Dissolve a vial of reagent 5 powder with 6 mL double distilled water, heat and dissolve in 90°C water bath. The prepared solution can be stored at 2-8°C with shading light for 3 days and be heated to dissolve in 90°C water bath when it is used again.
6. Preparation of reagent 6 working solution:
   Dissolve a vial of reagent 6 powder with 4 mL double distilled water and mix fully. The prepared solution can be stored at 2-8°C with shading light for 3 days.
7. Preparation of chromogenic working solution:
   Mix the reagent 5 working solution, reagent 6 working solution and reagent 7 at a ratio of 5:2:2. Prepare the fresh needed amount before use and the prepared solution can be stored with shading light.

8. Preparation of 40 μmol/L standard solution:

Mix the reagent 8 and double distilled water at a ratio of 1: 24. Prepare the fresh needed amount before use and the prepared solution can be stored with shading light.

▲ Sample preparation

1. Serum (plasma), urine and other liquid samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

3. Cell sample:

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 (1×10^6): normal saline (0.9% NaCl) (μL) =1: 300. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲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 (1.38-40 μmol/L).

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

Sample type	Dilution factor
Human serum	1
Human urine	20-30
Mouse serum	1-3
10% Rat brain tissue homogenate	1
Chicken plasma	1
10% Rat kidney tissue homogenate	1
Rat urine	20-30
10% Vegetable leaf tissue homogenate	1
1×10^6 Jurkat cell	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 40 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 8, 16, 20, 24, 28, 32, 40 μmol/L.Reference is as follows:

Number	Standard concentrations (μmol/L)	40 μmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	1000
B	8	200	800
C	16	400	600
D	20	500	500
E	24	600	400
F	28	700	300
G	32	800	200
H	40	1000	0

2. The measurement of samples

1. Incubation reaction

(1) Standard tube: Take a* μL of standard solution with different concentrations to 1.5 mL EP tubes.

Sample tube: Take a* μL of sample to 1.5 mL EP tubes.

[Note]: a* = Sample volume= Standard volume. For tissue and cell samples, a* is 100-200 μL. For serum or plasma, a* is 80-100 μL.

(2) Add 60 μL of enzyme working solution to each tube.

(3) Mix fully and incubate at 37°C with shading light for 60 min.

(4) Add 20 μL of reagent 3 to each tube.

(5) Add 10 μL of reagent 4 to each tube.

(6) Mix fully and stand at room temperature for 5 min, centrifuge at 10000 g for 10 min, then take the supernatant for detection.

2. Chromogenic reaction

(1) Standard well: Take 50 μL of chromogenic working solution to the corresponding wells.

Sample well: Take 50 μL of chromogenic working solution to the corresponding wells.

(2) Standard well:Take 120 μL of supernatant from standard tube to the corresponding wells.

Sample well:Take 120 μL of the supernatant from sample tube to the corresponding wells.

(3) Mix fully with microplate reader for 5 s and stand at room temperature for 5 min. Measure the OD value of each well at 530 nm with microplate reader.

▲Summary operation table

1. Incubation reaction

	Standard tube	Sample tube
Standard solution with different concentrations (μL)	a	
Sample (μL)		a
Enzyme working solution (μL)	60	60
Mix fully and incubate at 37°C with shading light for 60 min.		
Regent 3 (μL)	20	20
Regent 4 (μL)	10	10
Mix fully and stand at room temperature for 5 min, centrifuge at 10000 g for 10 min, then take the supernatant for detection.		

[Note]: a* = Sample volume= Standard volume. For tissue and cell samples, a* is 100-200 μL. For serum or plasma, a* is 80-100 μL.

2. Chromogenic reaction

	Standard well	Sample well
Chromogenic working solution (μL)	50	50
Supernatant of standard tube (μL)	120	
Supernatant of sample tube (μL)		120
Mix fully and stand at room temperature for 5 min. Measure the OD value of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample :

$$\text{NO content (μmol/L)} = (\Delta A_{530} - b) \div a \times f$$

2. Tissue and cell sample:

$$\text{NO content (μmol/gprot)} = (\Delta A_{530} - b) \div a \times f \div C_{pr}$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{530}: the absolute OD value of sample($OD_{sample} - OD_{blank}$)

f: Dilution factor of sample before tested.

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

Appendix I Performance characteristics

▲Example analysis

For rat brain tissue, take 100 μL of 10% rat brain tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0064x + 0.0025, the average OD value of the sample is 0.089, the average OD value of the blank is 0.060, the concentration of protein in sample is 4.74 gprot/L, and the calculation result is:

NO content (μmol/gprot) = (0.089 - 0.060 - 0.0025) ÷ 0.0064 ÷ 4.74 = 0.87 μmol/gprot