

## Nitric Oxide Assay Kit

Cat#:orb1499851 (manual)

Visible Spectrophotometer

Size: 50T/48S

### Product composition and storage conditions :

No.	Specifications	Storage Conditions
Extraction solution	50 ml ×1	4 °C
orb1499851- A	35 mL×1	4 °C away from light
orb1499851- B	3mL×1	4 °C
orb1499851- C	35mL×1	4 °C away from light
orb1499851- Standard	1ml×1 (10µmol/mL)	4 °C

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

### Introduction :

**Significance:** Nitric Oxide (NO) is widely distributed in the nervous system, circulatory system, respiratory system, digestive system, genitourinary system and Nitric Oxide tissues. As an information material between cells and in cells, it plays a role of signal transmission. It is a new type of biological messenger molecule, and plays an important role in the physiological and pathological processes of the body.

**Principle:** The Nitric Oxide readily oxidizes to form  $\text{NO}^{2-}$  in vivo or in aqueous solution. Under acidic conditions,  $\text{NO}^{2-}$  forms a Diazo with diazosalt sulfamide, which is further coupled to naphthyl vinyl diamine. The product has a characteristic absorption peak at 550 nm, the No content can be calculated by measuring the absorption value.

### Own supplies :

Balance, mortar or homogenizer, visible spectrophotometer, 1 ml glass cutlery, centrifuge, adjustable pipette , water bath, ice, distilled water

### Sample processing :

1. **Tissue:** According to the ratio of tissue mass (g) : Extraction solution volume (mL) : 1:5 ~ 10(about 0.1 g tissue should be taken and 1 mL Extraction solution should be added) , homogenized in ice bath, centrifuged at 10000 g 4°C for 15 min, the take the supernatant and put it on ice for testing.

2. **Bacteria or fungi:** According to the number of bacteria ( $10^4$ ), Extraction solution volume (mL) is 500 ~ 1000:1 ratio (5 million bacteria are recommended to add 1 mL Extraction solution) , the bacteria were broken by ultrasonic wave (ice bath, power 300 W, ultrasonic 3 s, interval 7 s, total time 3min) ; Then centrifuge for 15 min at 10000 g, 4°C, and take the supernatant and place it on ice for testing.

3. **Liquid sample (body fluids or culture fluids):** use and test directly.

### Measurement steps:

1. Preheat the visible spectrophotometer for at least 30 minutes, adjust the wavelength to 550nm .
2. Preparation of standard solution: Dilute the standard to 0.1,0.05,0.025,0.0125,0.00625,0.003125,0.0015625  $\mu\text{mol/mL}$  with distilled water.
3. Add the following reagents in sequence to the EP tube :

Reagent name	Blank tube ( $\mu\text{l}$ )	Measuring tube ( $\mu\text{l}$ )	Standard tube ( $\mu\text{l}$ )
Distilled water	1000		
Sample		1000	
Standard			1000
orb1499851- A		500	500
orb1499851- B	500		
orb1499851- C	500	500	500

Mix fully, stay at room temperature for 15min, the absorption value was determined at 550 nm and record A standard、A measuring、A blank, calculate  $\Delta A$  standard = A standard - A blank,  $\Delta A$  measuring = A measuring - A blank.

Note: blank tubes only need to be done 1-2 times.

### Nitric Oxide content calculation :

1. Draw the standard curve: Take the concentration of each standard solution as the x-axis, take the  $\Delta A$  standard as the y-axis as the standard curve, and obtain Equation  $y=kx+b$ ; Bring the  $\Delta A$  assay into the equation to find the x value.

2. NO content calculation

(1) Calculated by sample mass

$$\text{NO content ( } \mu\text{ mol/mg)} = x \times V \text{ sample} \div (V \text{ sample} \times C_{pr}) = x \div C_{pr}$$

(2) Calculated by protein concentration

$$\text{NO content ( } \mu\text{ mol/ g prot)} = x \times V \text{ sample} \div (W \times V \text{ sample} \div V \text{ sample total}) = x \div W$$

(3) Calculated by number of cells

$$\text{NO content ( } \mu\text{ mol/}10^4\text{ cell)} = x \times V \text{ sample} \div (V \text{ sample} \times \text{Number of cells} \div V \text{ sample total}) = x \div \text{Number of cells}$$

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(4) Calculated by liquid volume

$$\text{NO content ( } \mu \text{ mol/mL)} = x \times V_{\text{sample}} \div V_{\text{sample}} = x$$

(5)  $V_{\text{sample}}$ : sample volume added;  $V_{\text{sample total}}$ : Extraction solution added; Cpr: protein concentration of sample, mg/mL; W: sample mass, g; Number of cells:  $10^4$  (10000 cells) as a unit.

### Precautions:

1. Try to use fresh samples for testing, and take protective measures during operation.
2. If the detected OD value is outside the range of the standard curve, perform appropriate concentration or dilution on the sample and divide by the concentration multiple or multiply by the dilution multiple in the calculation formula.