

Nitric Oxide Assay Kit

Cat#: orb1499850 (manual)

Size: 100T/96S

Microassay

Product composition and storage conditions:

No.		Specifications	Storage Conditions
Extraction solution		100 ml ×1	4 °C
orb1499850- A	A1	Powder ×1	4 °C away from light, Add A2 in A1 to fully dissolve before use and use up within 3 days;
	A2	8mL×1	
orb1499850- B		1mL×1	4 °C
orb1499850- C		8mL×1	4 °C away from light
orb1499850- 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 NO₂⁻ in vivo or in aqueous solution. Under acidic conditions, NO₂⁻ 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 or microplate reader, micro quartz cuvette/96-wells plate, 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, then take the supernatant and put it on ice for testing.
2. Bacteria or fungi: According to the number of bacteria (10⁴), 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 or microplate reader 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 μmol/mL with distilled water.
3. Add the following reagents in sequence to the EP tube:

Reagent name	Blank tube (μl)	Measuring tube (μl)	Standard tube (μl)
Distilled water	100		
Sample		100	
Standard			100
orb1499850- A		50	50
orb1499850- B	50		
orb1499850- C	50	50	50

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 ΔA standard = A standard - A blank, ΔA 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 ΔA standard as the y-axis as the standard curve, and obtain Equation $y=kx+b$; Bring the ΔA assay into the equation to find the x value.

2. NO content calculation

(1) Calculated by sample mass

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

(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

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

(4) Calculated by liquid volume

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

Note: V_{sample} : sample volume added; $V_{\text{sample total}}$: Extraction solution added; C_{pr} : 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.