# Nitric Oxide Assay Kit

## Cat#:orb1499850 (manual)

**Microassay**

**Size: 100T/96S**

**Product composition and storage conditions :**

| No. | Specifications | Storage Conditions |
|---|---|---|
| Extraction solution | 100 ml ×1 | 4 ℃ |
| orb1499850- A | 8mL×1 | 4 °C away from light |
| orb1499850- B | 1mL×1 | 4 ℃ |
| 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 $NO^{2-}$ in vivo or in aqueous solution. Under acidic conditions, $NO^{2-}$ 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 or microplate reader, micro quartz cuvette/96-wells plate, 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℃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℃, 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
   NO content ( μ moL/mg) = x × V sample ÷ (V sample × Cpr) = x ÷ Cpr
   (2) Calculated by protein concentration
   NO content ( μ moL/ g prot) = x × V sample ÷ (W × V sample ÷ V sample total) = x ÷ W
   (3) Calculated by number of cells
   NO content （ μ mol/$10^4$ cell） = x × V sample ÷ (V sample × Number of cells ÷ V sample total) = x ÷ Number of cells

(4) Calculated by liquid volume

NO content（μmol/mL）= x × V sample ÷ V sample = x

(5) V sample：sample volume added；V 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.