



ISO 13485:2016 ISO 9001:2015

Ver.250201

## Nitric oxide (NO) Assay Kit

BC16005-01 (50 Tests/48 Samples)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

Nitric Oxide (NO) is a highly unstable biological free radical with small molecule, simple structure, NO is gas at room temperature, slightly soluble in water and fat-soluble, which can diffuse through biofilms quickly. As a biological messenger molecule, it plays a role in transmitting signals between cells and within cells. It is widely distributed in various tissues, especially neural tissues. It also plays a very important role in the nervous, circulatory, respiratory, digestive, and urogenital systems of the body. NO is easily oxidized to form NO<sub>2</sub> - and NO<sub>3</sub> in the body or in aqueous solution.

This method uses nitrate reductase to reduce NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> specifically. Under acidic conditions, NO<sub>2</sub><sup>-</sup> and Diazonium sulphonamide produce diazo compounds. The compounds could further couple with naphthyl vinyl diamine, the product has a characteristic absorption peak at 540 nm, and its absorbance value can be measured to calculate the NO content.

## Kit components

Reagent	Volume	Storage
Extract Solution	60mL	RT
Reagent I	30mL	RT
Standard	1mL 10 µmol/mL of NaNO <sub>2</sub>	RT

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer, 1ml glass cuvette and distilled water.

## Protocol

### I. Sample Preparation

**Tissue:** Prepare 10% tissue homogenate by adding 1mL Extract Solution to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 12000rpm, 4°C for 15 minutes and collect the supernatant on ice for testing.

**Bacteria or cells:** Harvest the cells and wash twice with PBS. Add 1mL Extract Solution to 10 million cells and ultrasonicate (300W, work time 3s/ interval 7s repeat for 3 minutes) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 12000rpm, 4°C for 15 minutes and collect the supernatant. The supernatant should be kept on ice.

#### Serum or Plasma

Directly use for the assay

### II. Assay procedure

- Preheat the spectrophotometer reader/ microplate reader for 30 min, adjust wavelength to 540 nm and set zero with distilled water.
- Dilute the standard using distilled water to a final concentration of 0.1 µmol/mL. Serially dilute from this standard to get 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 and 0.0015625 µmol/mL respectively.
- Add the reagents to microcentrifuge table as given below.

Reagent	Blank tube (B)	Test tube (T)	Standard Tube (S)
Distilled water	500μL	-	-
Standard solution	-	-	500μL
Sample	-	500μL	-
Reagent I	500μL	500μL	500μL

- Vortex to mix and incubate at room temperature for 10 minutes. Measure OD at 540nm in a 1mL glass cuvette. Record the absorbance as

Blank:  $A_B$

Test:  $A_T$

Standard:  $A_S$

Calculate:  $\Delta A_T = A_T - A_B$

$\Delta A_S = A_S - A_B$

## Calculations

- Prepare a standard graph with  $\Delta A_S$  in the y-axis and corresponding standard concentration in x-axis. Input  $\Delta A_T$  in the graph and extrapolate to obtain the concentration of unknown (x) in (μmol/mL).

- NO Content

- Protein concentration

$$\begin{aligned}\text{NO } (\mu\text{mol/mg protein}) &= x \times V_s \div (\text{Cpr} \times V_s) \\ &= x \div \text{Cpr}\end{aligned}$$

- Sample Weight

$$\begin{aligned}\text{NO } (\mu\text{mol/g weight}) &= x \times V_s \div (W \times V_s \div V_e) \\ &= x \div W\end{aligned}$$

- Cell Number

$$\begin{aligned}\text{NO } (\mu\text{mol}/10^4 \text{ cells}) &= x \times V_s \div (N \times V_s \div V_e) \\ &= x \div N\end{aligned}$$

- Serum/Plasma

$$\begin{aligned}\text{NO } (\mu\text{mol /mL}) &= x \times V_s \div V_s \\ &= x\end{aligned}$$

$V_s$ : Sample volume, 0.5 mL

$V_e$ : Extraction volume, 1 mL

Cpr: Sample protein concentration, mg/mL

W: Sample weight, g

N: The amount of bacteria or cells, 10<sup>4</sup>

T: Reaction time, 1 minute.

## Note:

- When the  $A_t$  is higher than 1, it is recommended to test the sample after dilution and multiply it by the dilution factor in the calculation formula.
- Try to use fresh samples for testing.