



ISO 13485:2016 ISO 9001:2015

Ver.250301

## Nitric oxide (NO) Assay Kit

BC16005-02 (100 Tests/96 Samples)

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

## Product Description

Nitric Oxide (NO) is a highly unstable biological free radical. 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  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in the body or in aqueous solution. This method uses nitrate reductase to reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  specifically. Under acidic conditions,  $\text{NO}_2^-$  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	110mL $\times$ 1	RT
Reagent I	12mL $\times$ 1	RT
Standard (10mM Sodium Nitrite)	1mL $\times$ 1	RT Keep away from light

## Reagents and Equipment Required but Not Provided

Constant temperature water bath/incubator, cooling centrifuge, microplate reader, 96 well flat bottom microplate, mortar/homogenizer, distilled water and microcentrifuge tubes.

## Protocol

### I. Sample Preparation

**Tissue:** Add 1mL Extract Solution to 0.2g tissue. Homogenate in ice and centrifuge at 12000  $\times$ g at 4°C for 15 minutes. Supernatant is used for the assay. Keep the supernatant on ice.

**Bacteria or cells:** Add 1mL Extract Solution to 10 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 300W, sonication 3s, interval 7s; repeat for 30 times). Centrifuge at 12000  $\times$ g at 4°C for 20 minutes. Supernatant is used for the assay. Keep the supernatant on ice.

**Serum/Plasma:** Use directly 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.
- Keep Reagent I on ice.
- Dilute the standard to 0.1, 0.05, 0.025, 0.00625, 0.003125, 0.0015625  $\mu\text{mol/mL}$  with distilled water
- Carry out the reaction in a 1.5mL micro centrifuge tube.

Reagent	Blank tube (B)	Test tube (T)	Standard Tube (S)
Distilled water	100 $\mu\text{L}$	-	-
Standard solution	-	-	100 $\mu\text{L}$
Sample	-	100 $\mu\text{L}$	-
Reagent I	100 $\mu\text{L}$	100 $\mu\text{L}$	100 $\mu\text{L}$

- Vortex to mix and incubate at room temperature for 10 minutes. Measure OD at 550nm 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 the concentration of standard in x-axis and corresponding absorbance ( $\Delta A_S$ ) in y-axis. Obtain the slope of the graph and substitute  $\Delta A_T$  into the equation to obtain sample concentration  $x$  ( $\mu\text{mol/mL}$ ).

### 1. *Protein concentration:*

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

### 2. *Sample weight:*

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

### 3. *Cells*

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

### 4. *Serum/Plasma:*

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

$V_s$  : Sample volume, 0.1 mL

$V_e$  : Extraction volume, 1 mL

Cpr : Sample protein concentration, mg/mL

W : Sample weight, g

N : The amount of bacteria or cells,  $10^4$

T : Reaction time, 1 minute.