(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

# NADH oxidase (NOX) Assay Kit

Catalog No.: BC00179

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:


✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com


**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

## Basic Information

| | |
|---|---|
| **Product Name** | NADH oxidase (NOX) Assay Kit |
| **Detection Method** | Colorimetric |
| **Sample Type** | Tissue, serum, plasma, cell, bacteria |
| **Assay Type** | Quantitative |
| **Detection Instrument** | Spectrophotometer (600 nm) |

## Product Introduction

NOX is widely present in animals, plants, microorganisms and cultured cells. In the presence of oxygen, it can directly oxidize NADH to NAD. This enzyme is not only involved in the regeneration of NAD, but also closely related to immune responses.

## Principle

NADH oxidase (NOX) catalyzes the oxidation of NADH to NAD. The oxidation of NADH is coupled with the reduction of 2,6-dichlorophenolindophenol (DCPIP), during which blue DCPIP is reduced to colorless DCPIP. The activity of NADH oxidase is calculated by measuring the reduction rate of blue DCPIP at 600 nm.

## Components

| No. | Components | Size | Notes |
|---|---|---|---|
| Reagent 1 | Liquid | 50 mL | Store at -20°C |
| Reagent 2 | Liquid | 10 mL | Store at -20°C |
| Reagent 3 | Liquid | 1 mL | Store at -20°C |
| Reagent 4 | Liquid | 50 mL | Store at 4°C |
| Reagent 5 | Liquid | 6 mL | Store at 4°C |

| | | | |
|---|---|---|---|
| Reagent 6 | Powder | 2 vials | Store at -20°C. Add 5 mL of distilled water to each vial before use. Aliquot any unused reagent and store at -20°C. Avoid repeated freeze-thaw cycles. |

## Storage

The kit has a validity period of 3 months.

## Preparation

Visible spectrophotometer, desktop centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, mortar, ice, distilled water.

- **Sample handling**

Isolation of cytoplasmic and mitochondrial proteins from tissues, bacteria or cells:

1. Accurately weigh 0.1 g of tissue or collect $5\times10^6$ cells, add 1 mL of Reagent 1 and 10 μL of Reagent 3, and homogenize using an ice-cold homogenizer or mortar and pestle.

2. Centrifuge the homogenate at 600 × g for 5 min at 4°C.

3. Discard the pellet, transfer the supernatant to a new centrifuge tube, and centrifuge at 11 000 × g for 10 min at 4°C.

4. The supernatant is the cytoplasmic protein fraction without mitochondria, which can be used to determine NOX leaked from mitochondria (this step is optional).

5. The pellet obtained in step 4 is the mitochondrial fraction. Add 200 μL of Reagent 2 and 2 μL of Reagent 3, and sonicate on ice (power 20% or 200 W, 3 s sonication, 10 s interval, repeated 30 cycles) for NOX activity assay.

Serum (plasma) samples: Assay directly.

## Operation process

Before formal determination, be sure to select 2-3 samples with expected large differences to conduct a preliminary experiment.

1. Preheat the spectrophotometer for at least 30 min, adjust the wavelength to 600 nm, and zero the instrument with distilled water.
2. Incubate Reagent 4, Reagent 5 and Reagent 6 at 37°C (for mammals) or 25°C (for other species) for 5 min.
3. Operation table:

| | **Assay tube** |
|---|---|
| Reagent 4 (μL) | 700 |
| Reagent 5 (μL) | 100 |
| Reagent 6 (μL) | 160 |
| Sample to be tested (μL) | 40 |
| Add the above reagents into a 1 mL cuvette, mix thoroughly, record the absorbance $A_1$ at 20 s and absorbance $A_2$ at 1 min 20 s at 600 nm, and calculate $\Delta A = A_1 - A_2$ (the reaction time is 1 minute). | |

## Calculation

1. Serum (plasma) NOX activity:

Definition: One enzyme activity unit is defined as a change of 0.01 in A600 per minute per mL of serum (plasma) in a 1 mL reaction system.

Calculation formula:

$$\text{NOX activity (U/mL)} = \Delta A \times V_{Total} \div V_{Sample} \div 0.01 \div T$$

2. NOX activity in tissues, bacteria or cells:

a. Calculated based on the protein concentration of the sample.

Definition: One enzyme activity unit is defined as a change of 0.01 in A600 per minute per mg of tissue protein in a 1 mL reaction system.

Calculation formula:

$$\text{NOX activity (U/mg)} = \Delta A \times V_{Total} \div (V_{Sample} \times Cpr) \div 0.01 \div T$$

b. Calculated based on sample fresh weight.

Definition: One enzyme activity unit is defined as a change of 0.01 in A600 per minute per gram of tissue in a 1 mL reaction system.

Calculation formula:

$$\text{NOX activity (U/g)} = \Delta A \times V_{Total} \div (W \times V_{Sample} \div V_{Extraction\ Solution}) \div 0.01 \div T$$

c. Calculated based on bacterial or cell density.

Definition: One enzyme activity unit is defined as a change of 0.01 in A600 per minute per 10,000 bacteria or cells in a 1 mL reaction system.

Calculation formula:

$$\text{NOX activity (U/10}^4\text{ cells)} = \Delta A \times V_{Total} \div (500 \times V_{Sample} \div V_{Extraction\ Solution}) \div 0.01 \div T$$

**Annotation:**

$V_{Total}$: Total volume of reaction system, 1 mL

$V_{Sample}$: Volume of sample added, 0.04 mL

$V_{Extraction\ Solution}$: Volume of extraction solution added, 0.202 mL

T: Reaction time, 1 min

Cpr: Sample protein concentration, mg/mL

W: Sample mass, g

500: Total number of cells or bacteria, $5\times10^6$

## Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.