

# NADH Oxidase (NOX) Assay Kit

(Cat/No.:BC114 Size:50T/48S)

## 1. Assay significance

NOX (EC 1.6.99.3) exists widely in animals, plants, microorganisms and cultured cells. NADH can be oxidized directly to NAD in the presence of oxygen. The NOX is not only involved in NAD regeneration, but also closely related to the immune response.

## 2. Assay principle (Colorimetric Method)

Nox Can oxidize NADH to NAD, and the oxidation of NADH is coupled with the reduction of 2,6 dichlorophenol indigo (DCPIP) . Blue DCPIP is reduced to colorless DCPIP, so we can measure the reduction rate of blue DCPIP at 600nm, and then we can calculate the activity of NOX.

## 3. Additional Materials Required

- ① ·Visible spectrophotometer, Activity (Wavelength:600nm)
- ② ·High speed refrigerated centrifuge and tubes, Water bath
- ③ ·Adjustable pipette (5-1000 $\mu$ l) and Tips
- ④ ·1mL glass cuvettes
- ⑤ ·Absolute ethanol and Distilled water

## 4. Composition

Reagent 1: Liquid 50mL $\times$ 1 bottle,store at -20 $^{\circ}$ C

Reagent 2: Liquid 10mL $\times$ 1 bottle,store at -20 $^{\circ}$ C

Reagent 3: Liquid 1mL $\times$ 1 bottle,store at -20 $^{\circ}$ C

Reagent 4: Liquid 50mL $\times$ 1 bottle,store at 4 $^{\circ}$ C

Reagent 5: Liquid 6mL $\times$ 1 bottle,store at 4 $^{\circ}$ C

Reagent 6: Powder $\times$ 2 bottles, store at -20 $^{\circ}$ .When used, add 5mL distilled water, mix well, An endless supply of the reagent can be kept at -20 $^{\circ}$ C but no repeated freezing and thawing

## 5. Sample preparation



Isolation of cytoplasmic protein and mitochondrial proteins from tissue, bacteria and cells

- ① Accurately weigh 0.1 g tissue or collect 5 million cells, add 1mL reagent I and 10uL Reagent III, and homogenize with ice bath homogenate or mortar.
- ② Centrifuged the homogenization at 4 °C for 5min at 600g。
- ③ Abandonment precipitate, and then remove the supernatant to another centrifuge tube and centrifuged at 4 °C for 10 min at 11000g.
- ④ The supernatant obtained by centrifugation is the cytoplasmic protein removed from mitochondria, It can be used to measure NOX leakage from mitochondria ( This step is optional )
- ⑤ The precipitate in step 4 is the Mitochondria. Add 200uL Reagent II and 2uL Reagent III, ultrasonic crushing (ice bath, power 20% or 200W, ultrasonic 3s, interval 10 seconds, repeat 30 times) for the determination of NOX activity.

Serum (Plasma) sample: Direct test.

## 6. Operation procedure

1、 The photometer is preheated for more than 30 minutes, the wavelength is adjusted to 600nm, and the distilled water is adjusted to zero。

2、 Sample determination

(1) Reagents IV, V and VI are incubated for 5 minutes at 37 °C (Mammal) or 25 °C

(other species)

(2) Add 40uL sample, 700uL reagent 4, 100uL Reagent 5 and 160uL Reagent 6 into 1 ml cuvette, and then mixed to record the absorbance values A1 and A2 after 1min 20s at 600nm, and then calculate  $\Delta A = A1 - A2$

## 7. Calculate of NOX

### 1、 NOX in serum (plasma)

**Unit definition:** A unit of enzyme activity was defined as A600 change 0.01 per minute per mL serum (plasma) per mL reaction system

$$\text{NOX (U/mL)} = \Delta A \times V_{\text{Total volume of reaction liquid}} \div V_{\text{sample}} \div 0.01 \div T = 2500 \times \Delta A$$

### 2、 NOX in tissue、 bacteria and cells

**(1) Calculated by sample protein concentration:**

**Unit definition:** An enzyme activity unit was defined as A600 change 0.01 per minute per mg tissue protein per mL reaction system.

$$\text{NOX (U/mg prot)} = \Delta A \times V_{\text{Total volume of reaction liquid}} \div (V_{\text{sample}} \times C_{\text{pr}}) \div 0.01 \div T = 2500 \times \Delta A \div C_{\text{pr}}$$

**This method requires self-determination of sample protein concentration.**

**(2) Calculated by sample fresh weight:**

**Unit definition:** An enzyme activity unit was defined as A600 changes 0.01 per minute per g tissue per mL reaction system .

$$\text{NOX (U/g tissue weight)} = \Delta A \times V_{\text{Total volume of reaction liquid}} \div (W \times V_{\text{sample}} \div V_{\text{Total volume of sample}}) \div 0.01 \div T = 505 \times \Delta A \div W$$

**(3) Calculated by bacteria or cell density**

**Unit definition:** An enzyme activity unit is defined as A600 change 0.01 per minute per 10,000 bacteria or cells per mL reaction system.

$$\text{NOX (U/10}^4 \text{ cell)} = \Delta A \times V_{\text{Total volume of reaction liquid}} \div (500 \times V_{\text{sample}} \div V_{\text{Total volume of sample}}) \div 0.01 \div T = 1.01 \times \Delta A$$

$V_{\text{Total volume of reaction liquid}}$ , 1mL;  $V_{\text{sample}}$ , 0.04mL  $V_{\text{Total volume of sample}}$ , 0.202 mL;

T: reaction time, 1 min;  $C_{\text{protein}}$ : Sample protein concentration, mg/mL

W: Sample quality, g; 500: Total number of cells or bacteria, 5 million