NADH Oxidase (NOX) Assay Kit

Catalog No.: abx298810

Size: 96 tests

Detection Range: 0.38 U/L – 22.09 U/L

Sensitivity: 0.38 U/L

Storage: Store all components at -20 °C in the dark.

Application: For detection and quantification of NOX activity in plant and animal tissue homogenates, and cell lysates.

Introduction

Abbexa's NOX Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NOX activity. NADH Oxidase in samples oxidizes NADH to NAD^+, while concurrently DCPIP is reduced, producing a color change from blue to colorless. The rate of color intensity loss can be measured at 600 nm, allowing calculation of NOX activity.

Kit Components

1. 96-well microplate
2. Extraction Solution A: 2 × 50 ml
3. Extraction Solution B: 30 ml
4. Inhibitor Solution: 2 × 0.8 ml
5. Buffer Solution: 20 ml
6. Substrate A: 2 × 1.2 ml
7. Substrate B: 2 vials
8. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (600 nm)
2. Double distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Sonicating water bath
6. Centrifuge

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20 °C or -80 °C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Weigh 0.1 g tissue, then add 0.9 ml of Extraction Solution A and 10 µl of Inhibitor Solution to homogenize the sample. Centrifuge at 600 x g at 4 °C for 5 minutes. Collect the supernatant and centrifuge at 12,000 x g at 4 °C for 15 minutes, then discard the supernatant and retain the precipitate. Add 200 µl of Extraction Solution B and 2 µl of Inhibitor Solution to the precipitate. Sonicate for 5 minutes, then centrifuge at 12,000 x g at 4 °C for 10 minutes. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately **(abx097193)**.
- **Cell lysates:** Add 1 x 10^6 cells to 0.4 ml of Extraction Solution A and 4 µl of Inhibitor Solution and homogenize. Centrifuge at 600 x g at 4 °C for 5 minutes. Collect the supernatant and centrifuge at 12,000 x g at 4 °C for 15 minutes, then discard the supernatant and retain the precipitate. Add 200 µl of Extraction Solution B and 2 µl of Inhibitor Solution to the precipitate. Sonicate for 5 minutes, then centrifuge at 12,000 x g at 4 °C for 10 minutes. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately **(abx097193)**.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Mouse liver tissue homogenate	5 – 10
10 % Cow liver tissue homogenate	5 – 8
10 % Mouse heart tissue homogenate	2 – 3
10 % Pig heart tissue homogenate	1 – 3
10 % Mouse kidney tissue homogenate	3 – 5
10 % Rat brain tissue homogenate	1
10 % Mouse muscle tissue homogenate	1
10 % *Epipremnum aureum* tissue homogenate	1

The sample diluent is Extraction Solution B

Note: Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

Bring all reagents to room temperature before use.

- **Substrate B:** Dissolve each vial with 1.2 ml of double distilled water, store on ice in the dark. The prepared solution may be aliquoted and stored at -20 °C in the dark for up to 3 days.

B. Assay Procedure

Note: The reaction rate is high, therefore we recommend measuring no more than 3 wells at one time.

1. Set the blank and sample wells, then add 20 µl of double distilled water or sample respectively.
2. Add 140 µl of Buffer Solution to each well.
3. Add 20 µl of Substrate A solution to each well.
4. Add 20 µl of Substrate B working solution to each well.
5. Mix thoroughly, then measure the OD (600 nm) of each well with a microplate reader at 30 seconds and 90 seconds. Record measurements for each well as A_1 and A_2 respectively.

C. Calculation of Results

1. Tissues and cell lysate samples:

One unit of NOX activity is defined as the quantity of NOX in 1 g of tissue or cell mitochondrial protein that catalyzes the reduction of 1 mmol of DCPIP per minute.

$$NOX\ activity\ (U/gprot) = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{21.8 \times 0.6} \times \frac{f \times 1000}{C_{prot} \times T}$$

where:

ΔA_{Sample}	Sample OD measurements $A_1 - A_2$
ΔA_{Blank}	Blank OD measurements $A_1 - A_2$
21.8	Molar absorption coefficient of DCPIP (L/mol/cm)
0.6	Optical path of microplate (cm)
C_{prot}	Concentration of mitochondrial protein in sample (gprot/L)
T	Time of reaction (1 min)
f	Dilution factor of sample
1000	Unit conversion (1 mmol/L = 1000 µmol/L)