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

Catalog No：E-BC-K806-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（590-610 nm）

Detection range：0.38-22.09 U/L

Elabscience®NADH Oxidase (NOX) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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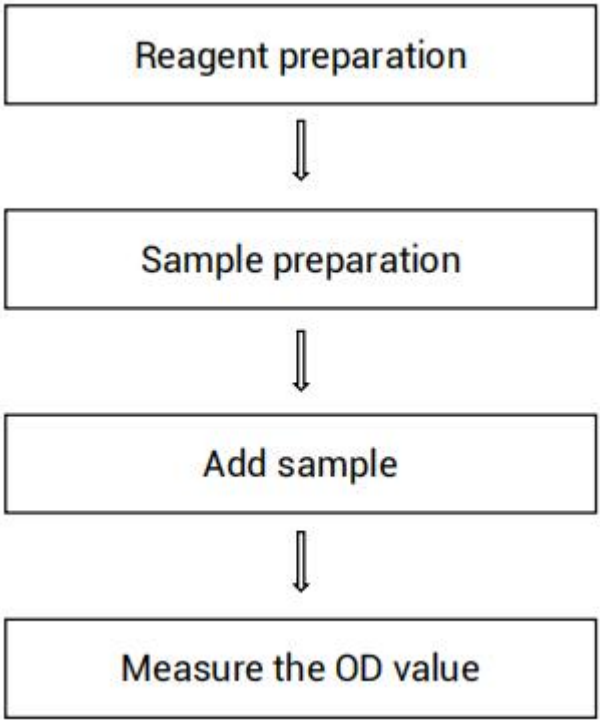
Assay summary



Intended use

This kit can measure NADH oxidase（NOX）activity in plant, animal tissue and cell samples.

Detection principle

NADH Oxidase （ NOX ） is widely found in the animals, plants, microorganisms and cultured cells, which can directly oxidize NADH to NAD^+ in the presence of oxygen, and reduce blue DCPIP to colorless DCPIP. The activity of NOX can be calculated by measuring the reduction rate of blue DCPIP at 600 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Extraction Solution B	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months
Reagent 3	Inhibitor	0.8 mL × 1 vial	0.8 mL × 2 vials	-20 °C, 12 months, shading light
Reagent 4	Buffer Solution	10 mL× 1 vial	20 mL × 1 vial	-20°C, 12 months
Reagent 5	Substrate A	1.2 mL× 1 vial	1.2 mL × 2 vials	-20°C, 12 months, shading light
Reagent 6	Substrate B	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Centrifuge, Microplate reader (590-610 nm, optimum wavelength: 600 nm）

Reagents:

Double distilled water

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of substrate B working solution：

Dissolve one vial of substrate B with 1.2 mL of double distilled water, mix well to dissolve. Aliquoted store at -20°C for 3 days protected from light.

③ The preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 200 μL of working solution (mix well 100 μL of substrate A and 100 μL of substrate B working solution). The working solution should be prepared on spot. Keep it on ice during use protected from light and used up within 0.5 h.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation
0.1 g).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 0.1 g tissue in 900 μL extraction solution A and 10 μL Inhibitor with a dounce homogenizer at 4℃.

④ Centrifuge at 600×g for 5 minutes to remove insoluble material. Discard the precipitate and take the supernatant. Centrifuge at 12000×g for 15 minutes, discard the supernatant and take the precipitate.

⑤ The precipitate was mixed with 200 μL of extraction solution B and 2 μL of Inhibitor, sonicated for 5 min, centrifuged at 12000×g at 4°C for 10 min. Collect supernatant and keep it on ice for detection.

⑥ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 400 μL extraction solution A and 4 μL Inhibitor with a dounce homogenizer at 4℃.

④ Centrifuge at 600×g for 5 minutes to remove insoluble material. Discard the precipitate and take the supernatant. Centrifuge at 12000×g for 15 minutes, discard the supernatant and take the precipitate.

⑤ The precipitate was mixed with 200 μL of extraction solution B and 2 μL of Inhibitor, sonicated for 5 min, centrifuged at 12000×g at 4°C for 10 min. Collect supernatant and keep it on ice for detection.

⑥ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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% Mouse heart tissue homogenate	2-3
10% Porcine heart tissue homogenate	1-3
10% Rat brain tissue homogenate	1
10% Mouse kidney tissue homogenate	3-5
10% Mouse muscle tissue homogenate	1
10% Bovine liver tissue homogenate	5-8
10%Epipremnum aureum tissue homogenate	1

Note: The diluent is extraction solution B. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

This reaction rate is relatively fast. It's better to measure no more than 3 sample wells at same time.

Operating steps

① Blank well: Add 20 μL of double distilled water to blank well.
Sample well: Add 20 μL of sample to sample well.

② Add 140 μL of buffer solution to each well.

③ Add 40 μL of working solution to each well.

④ Mix fully, measure the OD value of each well at 30 s and 1min 30s respectively at 600 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_1 - A_2$.

Calculation

The sample:

1. Tissue and cells sample:

Definition: The amount of NADH oxidase in 1 g tissue or cell mitochondrial protein per 1 minute that hydrolyze the substrate to produce 1 mmol oxidized DCPIP at room temperature is defined as 1 unit.

$$\frac{\text{NOX activity}}{(\text{U/gprot})} = \frac{(\Delta A_{\text{sample}} - \Delta A_{\text{blank}})}{21.8^{*} \times 0.6} \div C_{\text{pr}} \div T \times f \times 1000^{*}$$

[Note]

ΔA_{sample}: The change of OD value of sample ($A_1 - A_2$).

ΔA_{blank} : The change of OD value of blank ($A_1 - A_2$).

21.8*: The molar absorption coefficient of DCPIP, L/mol/cm.

0.6: The optical path of microplate, cm.

C_{pr}: The concentration of mitochondrial protein in sample, gprot/L.

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

1000*: 1 mol/L=1000 mmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse heart tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	13.50	18.40
%CV	4.3	4.0	3.7

Inter-assay Precision

Three mouse heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	13.50	18.40
%CV	6.8	7.0	7.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （U/L）	5.2	10.8	16.5
Observed Conc. （U/L）	5.1	11.1	17.2
Recovery rate(%)	99	103	104

Sensitivity

The analytical sensitivity of the assay is 0.38 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

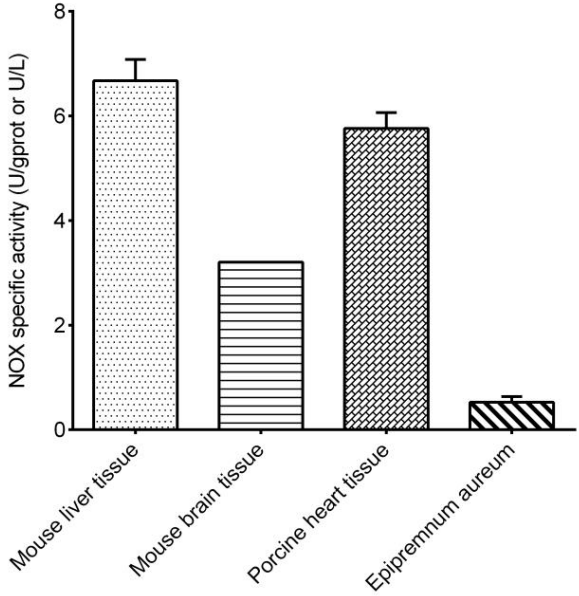
Example analysis：

For 10% mouse liver tissue mitochondria supernatant, dilute for 5 times, carry the assay according to the operation steps. The results are as follows:

The A_1 of the blank well is 0.763, the A_2 of the blank well is 0.757, The A_1 of the sample well is 0.439, the A_2 of the sample well is 0.178, the concentration of mitochondria protein in sample is 10.71 gprot/L and the calculation result is:

$$\frac{\mathrm{NOX}}{(\mathrm{U/gprot})} = ((0.439 - 0.246) - (0.763-0.757)) \div (21.8 \times 0.6) \div 10.71 \div 1 \times 5 \times 1000 = 6.67\mathrm{U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of mitochondria protein is 10.71 gprot/L, dilute for 4 times), 10% mouse brain tissue homogenate (the concentration of mitochondria protein is 4.65 gprot/L, dilute for 3 times), 10% porcine heart tissue homogenate (the concentration of mitochondria protein is 1.61 gprot/L, dilute for 3 times), and 10% Epipremnum aureum (the concentration of mitochondria protein is 0.51 gprot/L) according to the protocol, the result is as follows：


NOX specific activity (U/gprot or U/L)
8
6
4
2
0
Mouse liver tissue
Mouse brain tissue
Porcine heart tissue
Epipremnum aureum

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.