PRODUCT INFORMATION & MANUAL

NADH Oxidase/NOX Activity Assay Kit (Colorimetric)

NBP3-25867

For research use only.
Not for diagnostic or therapeutic procedures.

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NADH Oxidase/NOX Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25867

Method: Colorimetric method

Specification: 96T (Can detect 94 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.38 U/L

Detection range: 0.38-22.09 U/L

Average intra-assay CV (%): 4.0

Average inter-assay CV (%): 7.1

Average recovery rate (%): 102

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure NADH oxidase (NOX) activity in plant and 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	Specification	Storage
Reagent 1	Extraction Solution A	50 mL × 2 vials	-20℃ , 12 months
Reagent 2	Extraction Solution B	30 mL × 1 vial	-20℃ , 12 months
Reagent 3	Inhibitor	0.8 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Buffer Solution	20 mL × 1 vial	-20℃ , 12 months
Reagent 5	Substrate A	1.2 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 6	Substrate B	Powder × 2 vials	-20℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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.			

▲Materials prepared by users

Instruments

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ 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.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 6 working solution:

   Dissolve a vial of reagent 6 with 1.2 mL double distilled water and put it on ice box with shading light for use. The prepared solution can be divided into smaller packages at -20°C with shading light for 3 days.

▲ Sample preparation

1. Tissue sample:

   Accurately weigh 0.1 g tissue, then add 0.9 mL of reagent 1 and 10 μL of reagent 3 to homogenize the sample. Then centrifuge at 600 g for 5 min at 4 °C, discard the precipitate and take the supernatant. Then centrifuge at 12000 g for 15 min at 4°C, discard the supernatant and take the precipitate. The precipitate was mixed with 200 μL of reagent 2 and 2 μL of reagent 3, sonicated for 5 min, centrifuged at 12000 g at 4°C for 10 min. Then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

2. Cell sample:

   Mix 1×10^6 cells with 0.4 mL of reagent 1 and 4 μL of reagent 3 fully and homogenize. Then centrifuge at 600 g for 5 min at 4°C, discard the precipitate and take the supernatant. Then centrifuge at 12000 g for 15 min at 4°C, discard the supernatant and take the precipitate. The precipitate was mixed with 200 μL of reagent 2 and 2 μL of reagent 3, sonicated for 5 min, centrifuged at 12000 g at 4°C for 10 min. Then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.38-22.09 U/L).

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 reagent 2.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	S1	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S2	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S3	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S4	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S5	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S6	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
H	S7	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94

Note: A, blank wells; S1-S94, sample wells.

▲Detailed operation 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 reagent 4 to each well.

(3) Add 20 μL of reagent 5 to each well.

(4) Add 20 μL of reagent 6 working solution to each well.

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

▲Summary operation table

	Blank well	Sample well
Double distilled water (μL)	20	--
Sample (μL)	--	20
Regent 4 (μL)	140	140
Regent 5 (μL)	20	20
Reagent 6 working solution (μL)	20	20
Measure the OD value of each well at 30 s and 1min 30 s respectively at 600 nm with microplate reader, recorded as A_1, A_2, $\Delta A = A_1 - A_2$.		

▲Calculation

For tissue and cell 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.

$$\text{NOX activity (U/gprot)}$$

$$= (\Delta A_{sample} - \Delta A_{blank}) \div (21.8^* \times 0.6) \div C_{pr} \div T \times f \times 1000^*$$

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 mmol/L=1000 μmol/L.

Appendix I Data

▲Example analysis

For 10% mouse liver tissue mitochondria supernatant, dilute for 5 times, carry the assay according to the operation table. 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:

NOX activity (U/gprot) = ((0.439 - 0.246) - (0.763 - 0.757)) ÷ (21.8 × 0.6) ÷ 10.71 ÷ 1 × 5 × 1000 = 6.67 U/gprot