NADH Oxidase(NOX) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0576

Size: 100T/48S

Components:

Reagent I: Liquid 50 mL×1. Storage at 4℃.

Reagent II: Liquid 10 mL×1. Storage at 4℃.

Reagent III: Liquid 1 mL×1. Storage at -20℃.

Reagent IV: Liquid 35 mL×1. Storage at 4℃.

Reagent V: Liquid 5 mL×1. Storage at 4℃.

Reagent VI: Powder×2. Storage at -20℃. Add 4.5 mL of distilled water to each bottle before use. Keep the unused reagents in separate packages at -20℃.

Product Description:

NADH oxidase (NOX) (EC 1.6.99.3) exists widely in animals, plants, microorganisms and cultured cells, which can direct oxidation of NADH to NAD under oxygen. This enzyme is not only involved in the regeneration of NAD, but also closely related to immune response.

NOX can oxidize NADH to NAD, oxidation of NADH is coupled to the reduction phase of 2,6-dichlorophenol indigo (DCPIP) and the blue DCPIP is restored to colorless DCPIP. The NOX activity can be quantified by measuring the decrease in the color development at 600 nm.

Reagents and Equipment Required but Not Provided:

Microplate reader/spectrophotometer, water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

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

1) Collecting 0.1 g of tissue or 5 million cells, add 1 mL of Extract solution and 10 μL of Reagent III, grinding on ice with mortar/homogenizer.

2) Centrifuge at 600 ×g for 5 minutes at 4℃. Take the supernatant to other tube and centrifuge at 11000 g for 10 minutes at 4℃.

3) Take the supernatant to other tube. It's a cytoplasmic extract. The supernatant can be used to detect NOX activity.

4) The precipitate is mitochondria. Add 200 μL of Reagent II and 2 μL Reagent III to the sediment, blow repeatedly and mix well, used to detect the enzyme activity of NOX. It is also used for protein

concentration determination.

II. Determination

1) Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 600 nm, set zero with distilled water.

2) Preheat Reagent IV at 37℃ water bath.

3) Add the following reagents in micro glass cuvette/96 well flat-bottom plate:

Reagent	Test tube (T)	Control tube (C)
Reagent IV (μL)	175	175
Reagent V (μL)	25	25
Sample (μL)	10	10
Distilled water (μL)		40
Reagent VI (μL)	40	
Operate the above reagents in the micro glass cuvette/96 well flat-bottom plate in sequence. Mix thoroughly and timing after add Reagent VI, detect the absorbance at 600 nm at the time of 20s record as A_{T1} or A_{C1}. Then place dishes with the reaction solution in a 37℃ water bath for 1 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction at the time of 80s which record as A_{T2} or A_{C2}, $\Delta A_T=A_{T1}-A_{T2}$, $\Delta A_C=A_{C1}-A_{C2}$. $\Delta A=\Delta A_T-\Delta A_C$.		

III. Calculation:

1. Micro glass cuvette

Tissue protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 600 nm changed 0.01 in the reaction system per minute every milligram of protein.

NOX Activity (U/mg prot)=ΔA÷0.01×Vrv÷Cpr×Vs)÷T =2500×ΔA÷Cpr

Vrv: Total reaction volume, 0.25 mL;

Cpr: Supernatant sample protein concentration (mg/mL)

Vs: Sample volume (mL), 0.01 mL;

T: Reaction time (min), 1 minute;

2. 96 well flat-bottom plate

Tissue protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 600 nm changed 0.005 in the reaction system per minute every milligram of protein.

NOX Activity (U/mg prot)=ΔA÷0.005×Vrv÷Cpr×Vs)÷T =5000×ΔA÷Cpr

Vrv: Total reaction volume,0.25 mL;

Cpr: Supernatant sample protein concentration (mg/mL)

Vs: Sample volume (mL), 0.01 mL;

T: Reaction time (min), 1 minute;

Note:

1. Sample preparation processes should be operated at 0℃-4℃ to prevent denaturation and deactivation of enzyme.
2. Keep 37℃ of the react solution in cuvette, add 37℃ water to a beaker, put this beaker in 37℃ water bath and put the cuvette in this beaker.
3. It is better for two people to do this experiment at the same time, one for colorimetric and the other for timing to ensure the accuracy of the experimental results.
4. During the tests, keep Reagent VI on ice to avoid denaturation and deactivation.
5. The enzyme activity is calculated by reaction rate. When using 96 well flat-bottom plate, please control the number of samples measured at one time according to the operating speed.
6. It is recommended to use the sample protein concentration to calculate the enzyme activity. If the fresh weight of the sample is used for calculation, the enzyme activity of the cytoplasmic extract should be measured. The sum of supernatant and precipitation enzyme activity is the total enzyme activity.

7. Attachment: calculation formula of fresh weight of samples

Tissue weight:

a. Micro glass cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 600 nm changed 0.01 in the reaction system per minute every gram of tissue.

NOX Activity supernatant (U/g weight)=ΔA1÷0.01×Vrv÷(W÷Ve ×Vs)÷T =2525×ΔA1÷W

NOX Activity sediment (U/g weight)=ΔA2÷0.01×Vrv÷(W÷V_{ST}×Vs)÷T =505×ΔA2÷W

NOX Activity (U/g weight)=NOX supernatant+NOX sediment=2525×ΔA1÷W+505×ΔA2÷W

ΔA1: Supernatant absorbance;

ΔA2: Sediment absorbance;

Vrv: Total reaction volume, 0.25 mL;

Ve: Extract solution volume, 1.01 mL;

Vs: Sample volume (mL), 0.01 mL;

T: Reaction time (min), 1 minute;

W: Sample weight, g;

V_{ST}: Sediment heavy suspension volume, 0.202 mL.

b. 96 well flat-bottom plate

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance at 600 nm changed 0.005 in the reaction system per minute every gram of tissue.

NOX Activity supernatant (U/g weight) =ΔA1÷0.01×Vrv÷(W÷Ve ×Vs)÷T =5050×ΔA1÷W

NOX Activity sediment (U/g weight) =ΔA2÷0.01×Vrv÷(W÷V_{ST}×Vs)÷T =1010×ΔA2÷W

NOX Activity (U/g weight) =NOX supernatant +NOX sediment=5050×ΔA1÷W+1010×ΔA2÷W

ΔA1: Supernatant absorbance;

ΔA2: Sediment absorbance;

Vrv: Total reaction volume, 0.25 mL;
Vs: Sample volume (mL), 0.01 mL;
Ve: Extract solution volume, 1.01 mL;
V_{ST}: Sediment heavy suspension volume, 0.202 mL;
T: Reaction time (min), 1 minute;
W: Sample weight, g.

Experimental example:

1. 0.1g of lung is taken for sample treatment, and the operation is performed according to the determination steps with micro glass cuvette. $\Delta A1 = \Delta A_T - \Delta A_C$ = (0.8136-0.118) - (0.9216-0.8079) = 0.5819, $\Delta A2 = \Delta A_T - \Delta A_C$ = (0.8546-0.4736) - (0.9539-0.9121) = 0.3392 are measured

NOX supernatant (U/g mass) = 2525×ΔA1÷ W = 2525×0.5819 ÷ 0.1 = 14692.975

NOX precipitation (U/g mass) = 505×ΔA2 ÷ W = 505×0.3392 ÷ 0.1 = 1712.96

NOX (U/g mass) = NOX supernatant + NOX precipitation = 2525×ΔA1 ÷ W + 505×ΔA2÷ W

=2525×0.5819÷ 0.1 + 505×0.3392÷ 0.1 = 16405.935 U/g mass.

2. Take 0.1g of leaves for sample treatment, and operate according to the determination steps with micro glass cuvette. The results show that $\Delta A1 = \Delta A_T - \Delta A_C$ = (0.8518-0.7998) - (0.886-0.8831) = 0.0491, $\Delta A2 = \Delta A_T - \Delta A_C$ = (0.872-0.8296) - (0.916-0.9149) = 0.0413

NOX supernatant (U/g mass) = 2525×ΔA1 ÷ W = 2525×0.0491÷0.1 = 1239.775

NOX precipitation (U/g mass) = 505×ΔA2 ÷ W = 505×0.0413÷0.1 = 208.565

NOX (U/g mass) = NOX supernatant + NOX precipitation = 2525×ΔA1 ÷ W + 505×ΔA2 ÷ W

=2525×0.0491÷0.1+505×0.0413÷0.1 = 1448.34 U/g mass.

Recent Product Citations：

[1] Dou S, Liu S, Xu X, et al. Octanal inhibits spore germination of Penicillium digitatum involving membrane peroxidation[J]. Protoplasma, 2017, 254(4): 1539-1545.

[2] Liu P, Zhang H M, Hu K, et al. Sensory plasticity of carotid body is correlated with oxidative stress in paraventricular nucleus during chronic intermittent hypoxia[J]. Journal of cellular physiology, 2019, 234(8): 13534-13543.

[3] Yongtao Du,Mengjie Zhao,Changtao Wang,et al. Identification and characterization of GmMYB118 responses to drought and salt stress. BMC Plant Biology. December 2018;(IF3.67)

[4] Youqiang Xu,Chunyan Xu,Xiuting Li,et al. A combinational optimization method for efficient synthesis of tetramethylpyrazine by the recombinant Escherichia coli. Biochemical Engineering Journal. January 2018;(IF3.371)

[5] Weida Li,Kai Wang,Nanfang Jiang,et al. Antioxidant and antihyperlipidemic activities of purified polysaccharides from Ulva pertusa. Journal of Applied Phycology. April 2018;(IF4.784)

References：

[1] Kawai S, Mori S, Mukai T, et al. Cytosolic NADP phosphatases I and II from Arthrobacter sp. strain KM: implication in regulation of $NAD^+/NADP^+$ balance[J]. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of

Microorganisms, 2004, 44(3): 185-196.

Related Products：

NA0843/NA0601	Coenzyme I NAD(H) Content Assay Kit
NA0795/NA0554	NAD Kinase(NADK) Activity Assay Kit
NA0794/NA0553	NAD-Malate Dehydrogenase(NAD-MDH) Activity Assay Kit