NADH Oxidase(NOX) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0635

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Reagent I	Liquid 50 mL×1	2-8℃
Reagent II	Liquid 10 mL×1	2-8℃
Reagent III	Liquid 1 mL×1	-20℃
Reagent IV	Liquid 35 mL×1	2-8℃
Reagent V	Liquid 5 mL×1	2-8℃
Reagent VI	Powder×2	-20℃

Solution Preparation:

Reagent VI: Add 4.5mL distilled water before use, and dissolve it fully before use.The reagent that can not be used up is stored 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:

Visible spectrophotometer/microplate reader, desk centrifuge, mortar/homogenizer/cel ultrasonic crusher, micro glass cuvette/96 well plate, water bath, adjustable pipette, ice, distilled water.

Operation procedure:

I. Sample preparation:(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 Reagent I 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 procedure:

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

2) Preheat Reagent IV at 37℃ water bath.

3) Operation table:

Reagent name (μL)	Test tube (T)	Control tube (C)
Reagent IV	175	175
Reagent V	25	25
Sample	10	10
Distilled water	-	40
Reagent VI	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. Calculation formula of determination with micro glass cuvette

Calculate by sample 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. Calculation formula of determination with 96 well flat-bottom plate

Calculate by sample 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. Calculation formula of determination with 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 supernatant Activity (U/g weight)=ΔA1÷0.01×Vrv÷(W÷Ve ×Vs)÷T =2525×ΔA1÷W

NOX sediment Activity (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 supernatant Activity (U/g weight) =ΔA1÷0.005×Vrv÷(W÷Ve ×Vs)÷T =5050×ΔA1÷W

NOX sediment Activity (U/g weight) =ΔA2÷0.005×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. Take 0.1g of lung for sample treatment, and the operation is performed according to the determination steps with micro glass cuvette. ΔA1 = ΔA_T- ΔA_C = (0.8136-0.118) - (0.9216-0.8079) = 0.5819, ΔA2 = ΔA_T-ΔA_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] Zheng Q, Liu H, Zhang H, Han Y, Yuan J, Wang T, Gao Y, Li Z. Ameliorating Mitochondrial Dysfunction of Neurons by Biomimetic Targeting Nanoparticles Mediated Mitochondrial Biogenesis to Boost the Therapy of Parkinson's Disease. Adv Sci (Weinh). 2023 Aug;10(22):e2300758. doi: 10.1002/advs.202300758. Epub 2023 May 18. PMID: 37202595; PMCID: PMC10401119.

[2] Chen L, Tian Q, Shi Z, Qiu Y, Lu Q, Liu C. Melatonin Alleviates Cardiac Function in Sepsis-Caused Myocarditis via Maintenance of Mitochondrial Function. Front Nutr. 2021 Oct 11;8:754235. doi: 10.3389/fnut.2021.754235. PMID: 34708067; PMCID: PMC8542660.

[3] Shen T, Lyu D, Zhang M, Shang H, Lu Q. Dioscin Alleviates Cardiac Dysfunction in Acute Myocardial
Infarction via Rescuing Mitochondrial Malfunction. Front Cardiovasc Med. 2022 Mar 4;9:783426. doi: 10.3389/fcvm.2022.783426. PMID: 35310994; PMCID: PMC8931042.

[4] Zhang H, Yan X, Lin A, Xia P, Su Y. Inhibition of ghrelin activity by the receptor antagonist [D-Lys3]-GHRP-6 enhances hepatic fatty acid oxidation and gluconeogenesis in a growing pig model. Peptides. 2023 Aug;166:171041. doi: 10.1016/j.peptides.2023.171041. Epub 2023 Jun 8. PMID: 37301480.

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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.

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