Mitochondrial Respiratory Chain Complex I Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Catalog Number: BC0510

Size: 50T/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
Extract solution I	Liquid 75 mL×1	2-8℃
Extract solution II	Liquid 12 mL×1	-20℃
Reagent I	Liquid 50 mL×1	2-8℃
Reagent II	Powder ×2	2-8℃
Reagent III	Powder ×1	-20℃
Reagent IV	Powder ×2	-20℃

Solution Preparation:

1. **Reagent II**: The reagent is placed in a glass tube inside the bottle. Before use, take one bottle and add 2 mL of acetone to dissolve it thoroughly. It can be stored separately at 2-8℃ for one month;
2. **Reagent III:** Dissolve with 0.1mL of acetone before use. Acetone is volatile, pay attention to seal after use. The unused reagent can be stored at -20℃ for 2 months.
3. **Reagent III Working Solution:** Mix Reagent III: acetone = 10μL: 1mL (about 20T) according to the dosage before use, ready for use.
4. **Reagent IV:** Take a bottle and add 2.4mL of distilled water (about 30T) before use, prepare it immediately, dissolve it thoroughly, and store it at -20 ℃ for 1 month to avoid repeated freezing and thawing.
5. **Working Solution:** According to the amount of acetone: Reagent II: Reagent III working solution =250μL: 250μL: 500μL (about 10T) mixed for standby, ready for use.

Product Description:

Complex I (EC 1.6.5.3), also known as NADH CoQ reductase or NADH dehydrogenase, is widely present in the mitochondria of animals, plants, microorganisms, and cultured cells. It is the largest protein complex in the inner membrane of mitochondria. This enzyme catalyzes the transfer of a pair of electrons from NADH to CoQ, while also reducing O_2 to generate O^{2-}, which is the main site of O^{2-} production in the respiratory electron transport chain. Measuring the enzyme activity can not only reflect the status of the respiratory electron transfer chain (ETC), but also the status of reactive oxygen species (ROS) generation.

Complex I can catalyze the dehydrogenation of NADH to generate NAD+, and the oxidation rate of NADH is measured at 340nm to calculate the enzyme activity.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, desk centrifuge, water bath/constant temperature incubator, adjustable pipette, 1 mL quartz cuvette, mortar/homogenizer/cell ultrasonic crusher, acetone(>98%, AR), ice and distilled water.

Procedure:

1. Sample preparation:

1. Collecting 0.1g of tissue or 5 million cells, add 1mL of Extract solution I, grinding on ice with mortar/homogenizer (About 30 times).
2. Centrifuge at 600g at 4℃ for 10 minutes, discard the precipitate, and leave the supernatant. Centrifuge the supernatant again at 11000 ×g at 4℃ for 15 minutes to obtain the supernatant and precipitate.
3. The supernatant obtained from the previous step is the cytoplasmic extract, which can be used to determine the leakage of complex I from mitochondria (this step can be optional to determine the effectiveness of mitochondrial extraction).
4. Add 200μL of extraction solution one and 200μL of extraction solution two to the precipitate, sonicate (power 200W, sonication for 5s, interval 10s, repeated 15 times), for the determination of complex I enzyme activity and protein content.

II. Determination:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I at 37℃ for 15 minutes.
3. Add the following reagents in 1 mL quartz cuvette:

Reagent(μL)	Test tube (T)
Sample	50
Reagent I	770
Working Solution	100
Reagent IV	80
Immediately mix thoroughly and measure the absorbance value A1 at 340nm for 10 seconds. Quickly place it in a 37 ℃ water bath or constant temperature incubator for 1 minute, then remove it and quickly dry it to measure the absorbance value A2 at 1 minute and 10 seconds. Record the absorbance value A1 at 340nm for 10 seconds and the absorbance value A2 after 1 minute and 10 seconds. Calculate ΔA=A1-A2.	

III. Calculation:

Calculated based on sample protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every milligram tissue protein.

Complex I Activity (U/mg prot) =[ΔA×Vrv÷(ε×d) ×10^9] ÷(Vs×Cpr) ÷T =3215.43×ΔA÷Cpr

Vrv: Total reaction volume, 10^{-3} L;

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

Vs: Sample volume (mL), 0.05 mL;

Cpr: Sample protein concentration, mg/mL;
T: Reaction time (min), 1 minutes;
10^9: Unit conversion coefficient, 1mol=10^9nmol.

Note:

1. To ensure the accuracy of the experimental results, 1-2 samples need to be taken for preliminary experiments. If the measured absorbance value is too high (A1 > 1.5), the supernatant can be diluted with distilled water before measurement. When calculating the results, pay attention to multiplying by the dilution factor. If Δ A > 0.4, the sample needs to be diluted by an appropriate factor (multiplied by the corresponding dilution factor in the calculation formula); If Δ A is too small, sensitivity can be improved by increasing the sample volume added.
2. The protein concentration of the sample needs to be determined by oneself. Due to the presence of a certain concentration of protein (approximately 1mg/mL) in extraction solution one, it is necessary to subtract the protein content of the extraction solution itself (approximately 0.5mg/mL) when determining the protein concentration of the sample.
3. **It is recommended to use sample protein concentration to calculate enzyme activity.** Also attached are formulas for calculating sample quality and cell quantity
4. Attachment: calculation formula of sample weight: (the number of sample tests is 50T/24S)

A. Calculation of complex I activity in the supernatant::

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every gram of tissue.

Complex I Activity (U/g) =[ΔA1×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =3215.43×ΔA1÷W

ΔA1: Supernatant absorbance; Vrv: Total reaction volume, 10^{-3} L; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: Light path of cuvette, 1 cm; Ve: Extract solution volume,1 mL; Vs: Sample volume (mL), 0.05 mL; T: Reaction time (min), 1 minutes; W: Sample weight, g; 10^9: Unit conversion coefficient, 1mol=10^9nmol.

B. Calculation of the activity of complex I in precipitation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every gram of tissue.

Complex I Activity (U/g) =[ΔA2×Vrv÷(ε×d)×10^9]÷(W÷Vrs×Vs)÷T =1286.17×ΔA2÷W

ΔA2: Sediment absorbance; Vrv: Total reaction volume, 10^{-3} L;ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: Light path of cuvette, 1 cm; Vrs: Precipitate suspension volume (0.2mL Extract solution I +0.2mL Extract solution II), 0.4mL; Vs: Sample volume (mL), 0.05 mL; T: Reaction time (min), 1 minutes; W: Sample weight, g; 10^9: Unit conversion coefficient, 1mol=10^9nmol.

C. Total activity is the sum of Complex I activity.

The total activity of sample complex I is the sum of complex I activity in supernatant and

complex I activity in sediment.

Complex I Activity(U/g) =3215.43×ΔA1÷W+1286.17×ΔA2÷W

A. Attachment: calculation formula of cell number: (the number of sample tests is 50T/24S)

Supernatant:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every 10^6 cells.

Complex I Activity(U/10^6 cell) =[ΔA1×Vrv÷(ε×d)×10^9]÷(N÷Ve×Vs)÷T =3215.43×ΔA1÷N

ΔA1: Supernatant absorbance; Vrv: Total reaction volume, 10^{-3} L; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: Light path of cuvette, 1 cm; Ve: Extract solution volume,1 mL; Vs: Sample volume (mL), 0.05 mL; T: Reaction time (min), 1 minutes; N: cell number, 10^6; 10^9: Unit conversion coefficient, 1mol=10^9nmol.

B. Sediment:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADH per minute every 10^6 cells.

Complex I Activity(U/10^6 cell)=[ΔA2×Vrv÷(ε×d)×10^9]÷(N÷Vrs×Vs)÷T =1286.17×ΔA2÷N

ΔA2: Sediment absorbance; Vrv: Total reaction volume, 1 mL; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: Light path of cuvette, 1 cm; Vrs: Precipitate suspension volume (0.2mL Extract solution I +0.2mL Extract solution II), 0.4mL; Vs: Sample volume (mL), 0.05 mL; T: Reaction time (min), 1 minutes; N: cell number, 10^6; 10^9: Unit conversion coefficient, 1mol=10^9nmol.

D. Total activity is the sum of Complex I activity in supernatant and sediment.

The total activity of sample complex I is the sum of complex I activity in supernatant and complex I activity in sediment.

Complex I Activity(U/10^6 cell) =3215.43×ΔA1÷N+1286.17×ΔA2÷N.

Recent Products Citations:

[1] Zhou N, Qi H, Liu J, Zhang G, Liu J, Liu N, Zhu M, Zhao X, Song C, Zhou Z, Gong J, Li R, Bai X, Jin Y, Song Y, Yin Y. Deubiquitinase OTUD3 regulates metabolism homeostasis in response to nutritional stresses. Cell Metab. 2022 Jul 5;34(7):1023-1041.e8. doi: 10.1016/j.cmet.2022.05.005. Epub 2022 Jun 7. PMID: 35675826.

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[2] Eike L, Jakob M C, Julian D L, et al. Conformational changes in mitochondrial complex I from the thermophilic eukaryote Chaetomium thermophilum [J]. Science Advances, 2022, 8(47): 419-429.

[3] Pollard AK, Craig EL, Chakrabarti L. Mitochondrial Complex 1 Activity Measured by Spectrophotometry Is Reduced across All Brain Regions in Ageing and More Specifically in Neurodegeneration [J]. PLoS One, 2016, 11(6).

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BC3240/BC3245	Electron transport chain Complex III Activity Assay Kit
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