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Mitochondrial Respiratory(Electron transport) 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/Microplate reader

Cat No: AK0543-100T-96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract solution I	Liquid 75 mL×2	2-8℃
Extract solution II	Liquid 22 mL×1	-20℃
Reagent I	Liquid 20 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:** Before use, take a bottle and add 1 mL acetone, dissolve it fully. The unused reagent can be stored at 2-8℃ for 1 month. (One bottle of powder can make 100T. In order to prolong the use time of the kit, so give one more bottle of this product.)
2. **Reagent III:** Dissolve with 0.1 mL 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 = 5μL: 0.5mL (about 50T) according to the dosage before use, and ready for use.
4. **Reagent IV:** Before use, take one and add 1.6 ml distilled water to fully dissolve it (about 100T). The unused reagent can be stored at -20℃ for 1 month, avoid repeated freezing and thawing.
5. **Working Solution:** According to the amount of acetone: Reagent II: Reagent III working solution =250μL: 250μL: 500μL (about 50T) 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/microplate reader, desk centrifuge, water bath/constant temperature incubator, adjustable pipette, micro quartz cuvette/96 well flat-bottom UV plate, 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 600 ×g 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 or microplate reader 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 micro quartz cuvette/96 well flat-bottom UV plate:

Reagent	Test tube (T)
Sample (μL)	10
Reagent I (μL)	154
Working solution (μL)	20
Reagent IV (μL)	16
Mix thoroughly and timing, detect the absorbance at 340 nm at the time of 10 seconds record as A1. then try to react accurately in 37℃ environment for 1 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(1min10s). ΔA=A1-A2.	

I. Calculation:

1. Micro quartz cuvette

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,0.0002 L;

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

d: Light path of cuvette, 1 cm;

Vs: Sample volume (mL), 0.01 mL;

T: Reaction time (min), 1 minutes.

Cpr: Sample protein concentration (mg/mL);

10^9: Unit conversion factor，1mol=10^9nmol;

2. 96 well flat-bottom UV plate

The light path of the 96 well flat-bottom UV plate is 0.6 centimeter, change the light diameter in the formula of micro quartz cuvette from 1 cm to 0.6 cm.

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 100T/48S)

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.

D. 96 well flat-bottom UV plate

The light path of the 96 well flat-bottom UV plate is 0.6 centimeter, change the light diameter in the formula of micro quartz cuvette from 1 cm to 0.6 cm.

5. Attachment: calculation formula of cell number: (the number of sample tests is 100T/48S)

A. 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, 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; N: cell number, 10^6; 10^9: Unit conversion coefficient, 1mol=10^9nmol.

C. 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.

D. 96 well flat-bottom UV plate

The light path of the 96 well flat-bottom UV plate is 0.6 centimeter, change the light diameter in the formula of micro quartz cuvette from 1 cm to 0.6 cm.

References:

[1] Gadicherla A K, Stowe D F, Antholine W E, et al. Damage to mitochondrial complex I during cardiac ischemia reperfusion injury is reduced indirectly by anti-anginal drug ranolazine[J]. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2012, 1817(3): 419-429.

[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.