Mitochondrial Respiratory Chain Complex I Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC0515

Size:100T/96S

Components:

Extract solution: Liquid 75 mL×2. Storage at 4℃.

Reagent I: Liquid 20 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃, dissolve with 1 mL of acetone before use.

Reagent III: Powder×1. Storage at -20℃, dissolve with 1 mL of acetone, stored at -20℃ after sub-package. Dilute 100 times with acetone when the solution will be used.

Reagent IV: Powder×1. Storage at -20℃, add 2 mL of distilled water before use.

Working solution: Mix Reagent II and Reagent III by the ratio of 1:1 before use.

Product Description:

Mitochondrial Respiratory Chain Complex I (EC 1.6.5.3) same as NADH-CoQ reductase or NADH dehydrogenase, exists widely in mitochondria of animals, plants, microorganisms and cultured cells, which is the largest protein complex in inner mitochondrial membrane. This enzyme catalyzes a pair of electrons transfer from NADH to CoQ, reduce O_2 to O^{2-}, which is the main site of O^{2-} production on the respiratory electron transport chain. The enzyme activity of Complex I reflects the state of respiratory electron transport chain (ETC) and produce state of ROS.

Complex I catalyzes NADH to form NAD^+, the activity of this enzyme can be calculated by detecting the oxidation rate of NADH at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, micro quartz cuvette/96 well flat-bottom UV plate, water bath, desk centrifuge, adjustable pipette, mortar/homogenizer, acetone, ice and distilled water.

Procedure:

I. Complex extraction:

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

2) Centrifuge at 600 ×g for 10 minutes at 4℃.

3) Take the supernatant to another tube and centrifuge at 11000 g for 15 minutes at 4℃.

4) Supernatant is cytoplasmic extract. It can be used to detect Complex I that leaking from mitochondria, which shows the effect of mitochondrial extraction.

5) Add 400 μL of Extract solution into the sediment, splitting with ultrasonic (power 20%, work time 5s,

interval 10s, repeat 15 times), used to detect the enzyme activity of Complex I 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℃(mammal) or 25℃(other species) 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(10s). then try to react accurately in 37℃ (mammal) or 25℃ (other species) environment for 2 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(130s). ΔA=A1-A2	

II. Calculation:

1. Micro quartz cuvette

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 =1608×ΔA÷Cpr

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,0.0002 L;

Vs: Sample volume (mL), 0.01 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time (min), 2 minutes;

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. Take two or three different samples for prediction before test. Dilute supernatant with distilled water if the absorbance is higher than 1.5 or ΔA>0.4, multiply dilute times in the formula. While, increase the sample volume if ΔA is low.
2. The protein concentrate of the sample needs to be determined by yourself and our PC0020 BCA Protein Assay Kit is recommended. Since the Extract solution contains a relatively high concentration of protein, it is necessary to subtract the protein content of the Extract solution itself when determining the protein concentration of the sample.

3. It is recommended to use the sample protein concentration to calculate the enzyme activity. If the sample fresh weight is used to calculate, the enzyme activity of cytoplasmic extract needs to be measured, and the sum of supernatant and precipitation enzyme activity is the total enzyme activity.
4. The reagent in this kit is enough to complete 100 tube reaction.
5. Attachment: calculation formula of sample weight: (the number of sample tests is 100T/96S)

A. Supernatant:

1) Sample weight:

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 =1608×ΔA1÷W

ΔA1: Supernatant absorbance;
Vrv: Total reaction volume, 2×10^{-4} L;
ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1cm;
Ve: Extract solution volume,1 mL;
Vs: Sample volume (mL), 0.01 mL;
T: Reaction time (min), 2 minutes;
W: Sample weight, g.

B. Sediment:

1) Sample weight:

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 tissue.

Complex I Activity(U/g)=[ΔA2×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =643×ΔA2÷W

ΔA2: Sediment absorbance;
Vrv: Total reaction volume, 2×10^{-4} L;
ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
Ve: Suspended sediment volume, 0.4 mL;
Vs: Sample volume (mL), 0.01 mL;
T: Reaction time (min), 2 minutes;
W: Sample weight, g.

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.

1) Sample weight:

Complex I Activity(U/g) = 1608×ΔA1÷W +643×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.

Experimental example:

1. Take 0.1g of mouse lung for sample treatment, and operate according to the determination steps. Using micro quartz cuvette, we measured ΔA1 = A1-A2 = 1.4505-1.2163 = 0.2342, ΔA2 = A1-A2=1.499-1.398 = 0.101

The activity of complex I in the supernatant (U/g mass) =1608×ΔA1÷ W = 1608 × 0.2342 ÷0.1 = 3765.936 U/g mass

The activity of complex I in the precipitation (U/g mass) = 643 × ΔA2 ÷ W = 643 × 0.101 ÷ 0.1 = 649.43 U/g mass

Then complex I (U/g mass) = 1608 × ΔA1 ÷ W + 643 × ΔA2÷ W = 1608 × 0.2342÷0.1 + 643 × 0.101÷0.1 = 4415.366 U/g mass.

2. Take 0.1g of Magnolia for sample treatment, the supernatant and precipitation are diluted 4 times, according to the determination steps, using micro quartz cuvette to measure ΔA1 = A1-A2 = 1.3518-1.3348 = 0.017, ΔA2 = A1-A2 = 1.4269-1.4115 = 0.0154

The activity of complex I in the supernatant (U/g mass) = 1608× ΔA1 ÷ W × 4(dilution ratio) = 1608 × 0.017 ÷ 0.1 × 4 = 1093.44 U/g mass

The activity of complex I in the precipitation (U/g mass) = 64 ×ΔA2 ÷ W× 4 (dilution ratio) = 643× 0.0154 ÷ 0.1×4 = 396.088 U/g mass

Then complex I (U/g mass) = 1608 × ΔA1÷ W×4 (dilution multiple) +643 ×ΔA2 ÷W× 4 (dilution multiple) = 1608 × 0.017 ÷0.1×4 + 643×0.0154÷ 0.1 × 4 = 1489.528 U/g mass.

Recent Products Citations:

[1] Huazhang Zhu,Weizhen Zhang,Yingying Zhao,et al. GSK3β-mediated tau hyperphosphorylation triggers diabetic retinal neurodegeneration by disrupting synaptic and mitochondrial functions. Molecular Neurodegeneration. November 2018;(IF8.274)

[2] Liuqin He,Haiwen Zhang, Xihong Zhou. Weanling Offspring of Dams Maintained on Serine-Deficient Diet Are Vulnerable to Oxidative Stress. Oxidative Medicine and Cellular Longevity. September 2018;(IF4.868)

[3] Qiuli OuYang,Nengguo Tao,Miaoling Zhang. A Damaged Oxidative Phosphorylation Mechanism Is Involved in the Antifungal Activity of Citral against Penicillium digitatum. Frontier in Immunology. February 2018;(IF4.259)

[4] Wang M, Zhang Y, Xu M, et al. Roles of TRPA1 and TRPV1 in cigarette smoke-induced airway epithelial cell injury model[J]. Free Radical Biology and Medicine, 2019, 134: 229-238.

[5] Bao Z, Xu X, Chao H, et al. ERK/Nrf2/HO-1 pathway-mediated mitophagy alleviates traumatic brain injury-induced intestinal mucosa damage and epithelial barrier dysfunction[J]. 2017.

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.

Related Poducts:

BC3230/BC3235	Electron transport chain Complex II Activity Assay Kit
BC3240/BC3245	Electron transport chain Complex III Activity Assay Kit
BC0940/BC0945	Electron transport chain Complex IV Activity Assay Kit
BC1440/BC1445	Electron transport chain Complex V Activity Assay Kit