Mitochondrial Respiratory Chain Complex II Activity Assay Kit

(Succinate-Co-Enzyme Q Reductase Activity)

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

Operation Equipment: spectrophotometer/ microplate reader

Cat No: BC3235

Size:100T/96S

Components:

Extract solution I: Liquid 65mL×2. Store at 2-8℃.

Extract solution II: Liquid 22mL×1. Store at -20℃.

Reagent I: Liquid 16mL×1. Store at 2-8℃.

Reagent II: Powder×1. Store at -20℃. Dissolve with 0.1mL acetone to one Reagent II before use. It can be stored at -20℃ for 8 weeks.

Reagent II-working solution: Reagent II: acetone = 10μL : 1mL (about 100T) is mixed and prepared according to the sample numbers before use.

Reagent III: Powder×2. Store at 2-8℃. Dissolve with 1mL acetone to one Reagent III before use. It can be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing.

Reagent IV: Liquid 2.5ml×1. Store at 2-8℃.

Reagent V: Liquid 1.5ml×1. Store at 2-8℃.

Working solution: Mix acetone: Reagent II-working solution: Reagent=0.25mL： 0.5mL：0.25mL（1mL，about 50T）according to sample numbers before use.

Product Description:

Mitochondrial complex II is the same as succinate-Co-enzyme Q reductase, which exists widely in mitochondria of animal, plant, microorganisms and cultured cells. It catalyzes succinic acid to form fumaric acid, reduce FAD to form $FADH_2$. The $FADH_2$ reduce oxidized CoQ to form reduced CoQ, which is a branch of respiratory electron transport chain.

CoQ that a catalytic product of complex II could reduce 2,6-dichloroindophenol, which has absorbance at 605 nm, the activity of enzyme can be calculated by detecting the decrease rate of 2, 6-dichlorindolepheno.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro glass cuvette/96 well plate, water bath, desk centrifuge, transferpettor, mortar/homogenizer/cell ultrasonic crusher, acetone（>98%，AR）, ice and distilled water.

Procedure:

I. Complex II extraction:

1) Collecting 0.1g of tissue or 5 million cells, add 1ml of extract solution and grind on ice with mortar/homogenizer;

2) centrifuge at 600g and 4℃ for 10min. Discard the precipitate and transfer supernatant to another tube, centrifuge at 11000g and 4℃ for 15min;
3) The supernatant, i.e. cytoplasmic extract, can be used to determine the complex II leaking from mitochondria, this step can shows the effect of mitochondrial extraction;
4) Add 200μL Extract solution I amd 200μL Extract solution II to sediment, splitting with ultrasonication (power 200W, work time 5s， interval 10s， repeat 15 times), used to detect Complex II activity and protein content.

II. Determining step

1) Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 605 nm, set spectrophotometer to zero with distilled water.
2) Preheat Reagent I at 37℃ for 15 minutes.
3) Add the following reagents in micro glass cuvette/ 96 well flat-bottom plate:

Reagent (μL)	Test tube (A1)
Sample	10
Reagent I	140
Working solution	20
Reagent IV	20
Reagent V	10
Add the above reagent to the micro glass cuvette/ 96 well plate, mix thoroughly, detect absorbance at 10s (A1). Put cuvette and react solution together in 37℃ water bath for 5 min, then take cuvette quickly, dry and detect absorbance at 5 min (A2), ΔA=A1-A2.	

III. Calculation:

1. Ultra-micro cuvette

Protein concentration (need to detect protein concentration by yourself)

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1nmol of 2, 6-dichlorindolepheno per mg of tissue protein in every minute.

Complex II Activity (U/mg prot)=[ΔA×Vrv÷(ε×d)×10^9]÷(Vs×Cpr)÷T =190.5×ΔA÷Cpr

ε: 2, 6-dichlorindolepheno molar extinction coefficient, 2.1×10^4L/mol/cm;

d: light path of cuvette, 1cm;

Vrv: total reaction volume,2×10^{-4} L;

Vs: sample volume, 0.01 mL;

Cpr: sample protein concentration, mg/mL;

T: reaction time, 5 min;

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

2. 96 well plate

Change d-1cm in the above formula to d-0.6cm (light path of 96 well flat- bottom plate) for calculation.

Note:

1. Take two or three different samples for prediction before test to ensure the accuracy of experimental results. Dilute supernatant with distilled water if absorbance is higher than 1.2. Dilute sample with distilled water if ΔA>0.4, multiply dilute times in the formula. Increase sample volume if ΔA is low.
2. Since the extract contains a relatively high concentration of protein, it is necessary to subtract the protein content of the extract itself（Extract solution I +Extract solution II）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. It's enough for 100 tube reactions.
5. Attachment: Sample weight (100T/48S)

A. Supernatant:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1nmol of 2, 6-dichlorindolepheno in 1min every gram of tissue weight.

Complex II Activity(U/g weight)=[ΔA1×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =190.5×ΔA1÷W

ΔA1: supernatant absorbance;
Vrv: total reaction volume,2×10^{-4} L;
ε: 2, 6-dichlorindolepheno molar extinction coefficient, 2.1×10^4L/mol/cm;
d: light path of cuvette, 1cm;
Ve: extract solution volume,1mL;
Vs: sample volume (mL), 0.01 mL;
T: reaction time (min), 5 min;
W: sample weight, g.

B. Sediment:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1nmol of 2, 6-dichlorindolepheno in 1min every gram of tissue weight.

Complex II Activity(U/g)=[ΔA2×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =76.2×ΔA2÷W

ΔA2: sediment absorbance;
Vrv: total reaction volume,2×10^{-4} L;
ε: 2, 6-dichlorindolepheno molar extinction coefficient, 2.1×10^4L/mol/cm;
d: light path of cuvette, 1cm;
Ve: sediment resuspended volume,0.4 mL;
Vs: sample volume (mL), 0.01 mL;
T: reaction time (min), 5 min;

W: sample weight, g.

C. Total activity is the sum of Complex IIactivity in supernatant and sediment.

Complex II Activity(U/g)=190.5×ΔA1÷W +76.2×ΔA2÷W.

D. 96 well plate

Change d-1cm in the above formula to d-0.6cm for calculation.

Recent Product Citations:

[1] hou Y, Tang J, Lan J, Zhang Y, Wang H, Chen Q, Kang Y, Sun Y, Feng X, Wu L, Jin H, Chen S, Peng Y. Honokiol alleviated neurodegeneration by reducing oxidative stress and improving mitochondrial function in mutant SOD1 cellular and mouse models of amyotrophic lateral sclerosis. Acta Pharm Sin B. 2023 Feb;13(2):577-597. doi: 10.1016/j.apsb.2022.07.019. Epub 2022 Aug 10. PMID: 36873166; PMCID: PMC9979194.

[2] Xin J, Zhu B, Wang H, Zhang Y, Sun N, Cao X, Zheng L, Zhou Y, Fang J, Jing B, Pan K, Zeng Y, Zeng D, Li F, Xia Y, Xu P, Ni X. Prolonged fluoride exposure induces spatial-memory deficit and hippocampal dysfunction by inhibiting small heat shock protein 22 in mice. J Hazard Mater. 2023 Aug 15;456:131595. doi: 10.1016/j.jhazmat.2023.131595. Epub 2023 May 7. PMID: 37224709.

[3] Zhang Y, Zhang Y, Lei Y, Wu J, Kang Y, Zheng S, Shao L. MDM2 upregulation induces mitophagy deficiency via Mic60 ubiquitination in fetal microglial inflammation and consequently neuronal DNA damage caused by exposure to ZnO-

[4] NPs during pregnancy. J Hazard Mater. 2023 Sep 5;457:131750. doi: 10.1016/j.jhazmat.2023.131750. Epub 2023 Jun 1. PMID: 37315416.

[5] Zhao S, Hong Y, Liang YY, Li XL, Shen JC, Sun CC, Chu LL, Hu J, Wang H, Xu DX, Zhang SC, Xu DD, Xu T, Zhao LL. Compartmentalized regulation of NAD+ by Di (2-ethyl-hexyl) phthalate induces DNA damage in placental trophoblast. Redox Biol. 2022 Sep;55:102414. doi: 10.1016/j.redox.2022.102414. Epub 2022 Jul 20. PMID: 35926314; PMCID: PMC9356100.

[6] Lv Q, Han X, Ni J, Ma Q, Dai R, Liu J, Liu J, Zhai Y, Shen Q, Sun L, Liu H, Rao J, Xu H. Anti-ANGPTL3-FLD monoclonal antibody treatment ameliorates podocyte lesions through attenuating mitochondrial damage. Cell Death Dis. 2022 Oct 13;13(10):867. doi: 10.1038/s41419-022-05313-7. PMID: 36229446; PMCID: PMC9562403.

References:

[1] Mühling J, Tiefenbach M, López-Barneo J, et al. Mitochondrial complex II participates in normoxic and hypoxic regulation of α-keto acids in the murine heart[J]. Journal of molecular and cellular cardiology, 2010, 49(6): 950-961.

Related Products:

BC0510/BC0515	Mitochondrial Respiratory Chain Complex I Activity Assay Kit
BC3240/BC3245	Mitochondrial Respiratory Chain Complex III Activity Assay Kit
BC0940/BC0945	Mitochondrial Respiratory Chain Complex IV Activity Assay Kit
BC1440/BC1445	Mitochondrial Respiratory Chain Complex V Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com