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

Cat No: BC3230

Size:50T/48S

Components:

Extract solution: Liquid 80mL×1. Store at 2-8℃.

Reagent I: Liquid 40mL×1. Store at 2-8℃.

Reagent II: Liquid 0.1mL×1. Store at -20℃. Mix Reagent II: acetone = 0.01: 1mL (about 20T) according to the sample volume before use.

Reagent III: Powder×2. Store at 2-8℃. Dissolve with 2mL 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 5ml×1. Store at 2-8℃.

Working solution: Mix Reagent II and Reagent III by ratio of 1:1 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, water bath, desk centrifuge, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1ml glass cuvette, **acetone**, ice and distilled water.

Procedure:

I. Complex II extraction:

1) Collecting 0.1g of tissue or 5 million cells, add 1ml 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 show the effect of mitochondrial extraction;

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4) Add 400 μL extraction solution 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 for 30 minutes, adjust the wavelength to 605 nm, set zero with distilled water.

2) Preheat Reagent I at 37℃(mammal cell) or 25℃(other species) for 15 minutes.

3) Add the following reagents in 1ml glass cuvette:

Reagent (μL)	Test tube (A1)
Sample	50
Reagent I	750
Working solution	100
Reagent IV	100
Add the above reagent to the 1ml glass cuvette, mix thoroughly, detect absorbance at 10s (A1). Put cuvette and react solution together in 37℃(mammal) or 25℃(other species) water bath for 2 min, then take cuvette quickly, dry and detect absorbance at 2 min (A2), ΔA=A1-A2.	

III. Calculation:

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 =476.2×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,1mL;

Vs: sample volume (mL), 0.05 mL;

Cpr: sample protein concentration (mg/mL);

T: reaction time (min), 2 min;

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

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 1.5. 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 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 50 tube reactions.

5. Attachment: Sample weight (50T/24S)

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)=[ΔA1×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =476.2×ΔA1÷W

ΔA1: supernatant absorbance;

Vrv: total reaction volume,1mL;

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), 0.05 mL;

T: reaction time (min), 2 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 =190.5×ΔA2÷W

ΔA2: sediment absorbance;

Vrv: total reaction volume,1mL;

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), 0.05 mL;

T: reaction time (min), 2 min;

W: sample weight, g.

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

Complex II Activity(U/g) =476.2×ΔA1÷W +190.5×ΔA2÷W.

Experimental example:

1. Take 0.1g of rabbit liver sample, add 1 mL of Extract solution, grind and centrifuge the homogenate, and operate according to the determination steps. ΔA1 = A1-A2 = 1.134-1.054 = 0.08 in the supernatant, and ΔA2 =A1-A2 = 1.371-1.347 = 0.024 in the precipitation.

The activity of complex II in the supernatant (U/g mass) = 476.2 × 0.08÷0.1 = 380.96 U/g mass

The activity of complex II in the precipitation (U/g mass) = 190.5×0.024÷0.1 = 45.72 U/g

mass

Complex II Activity(U/g mass) = 476.2×0.08÷0.1 + 190.5×0.024 ÷ 0.1 = 426.68 U/g mass.

Recent Product Citations:

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

[2] 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.

[3] 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] 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

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