SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Electron transport chain Complex Ⅱ Activity Assay Kit

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

Operation Equipment: spectrophotometer/ microplate reader

Cat No: AK0367

Size:100T/96S

Components:

Extract solution: 80ml×2. Storage at 4℃ .

Reagent 1: 40ml×1. Storage at 4℃ .

Reagent 2: Powder×1. Storage at -20℃, dissolve with 1ml of acetone. The dissolved reagent 2 can be stored at -20℃ after dispensing. Dilute 100 times when use

Reagent 3: Powder×1. Storage at -20℃, dissolve with 2ml of acetone before use.

Reagent 4: 2.5ml×1. Storage at 4℃ .

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 flat-bottom plate, water bath, desk centrifuge, transferpettor, acetone, mortar/homogenizer, acetone, ice and distilled water.

1. **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 400ul extraction solution to sediment, splitting with ultrasonication (power 20%, work time 5s，interval 10s ，repeat 15 times), used to detect Complex Ⅱ activity and protein content.

Determining step

1. Preheat spectrophotometer/ microplate for 30min, adjust the wavelength to 605 nm, set the counter to zero with distilled water.

2. Sample determination

1) Making working solution: mix reagent 2 and reagent 3 as ratio of 1:1 before use. Prepared when the solution will be used.

2) Preheat reagent1 at 37℃(mammal cell) , 25℃(other species) for 15 min.

3) Add the following reagents in micro glass cuvette/ 96 well plate:

Reagent name (uL)	Test tube A1
Sample	10
Reagent 1	150
Working solution	20
Reagent 4	20
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℃(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	

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(nmol/min/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,2×10^{-4} L;

Vs: sample volume (mL), 0.01 mL;

Cpr: sample protein concentration (mg/mL);

T: reaction time (min), 2 min;

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 1.5. Dilute sample with distilled water if ΔA>0.4, multiply dilute times in the formula. Increase sample volume if ΔA is low.

2. Detect sample protein concentrate by yourself, you can use Solarbio (PC0020 BCA Protein Assay Kit). Because protein is contained in the extract, the protein content of the extract itself should be subtracted 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)

1) Supernatant:

2) 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,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), 2 min;

W: sample weight, g.

3) Sediment:

4) 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,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), 2 min;

W: sample weight, g.

5) **Total activity is the sum of Complex Ⅱ activity in supernatant and sediment.**

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

6) 96 well plate

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

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 =0.9851-0.7974=0. 1877 in the supernatant, and ΔA2 =A1-A2 =1.2561-0.9659=0.2902 in the precipitation.

The activity of complex II in the supernatant (U/g mass) = 476.2×ΔA1÷W = 476.2×0. 1877÷0. 1=893.8274 U/g mass

The activity of complex II in the precipitation (U/g mass) = 190.5×ΔA2÷ W = 190.5×0.2902÷0. 1= 552.831 U/g mass

Complex II (U/g mass) = 476.2 × ΔA1÷W + 190.5×ΔA2÷W = 1446.658 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：

AK0544/AK0543	Electron Transport Chain Complex I Activity Assay Kit
AK0366/AK0365	Electron transport chain Complex Ⅲ Activity Assay Kit
AK0274/AK0273	Electron transport chain Complex Ⅳ Activity Assay Kit
AK0263/AK0021	Electron transport chain Complex Ⅴ Activity Assay Kit