

## **Electron transport chain Complex II Activity Assay Kit**

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

**Operation Equipment:** spectrophotometer

**Cat No:** NA0639

**Size:**50T/48S

### **Components:**

Extract solution: 80ml×1. Storage at 4°C.

Reagent 1: 40ml×1. Storage at 4°C.

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

Reagent 3: Powder×1. Storage at 4°C, dissolve with 3ml of acetone before use.

Reagent 4: 5ml×1. Storage at 4°C.

### **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<sub>2</sub>. The FADH<sub>2</sub> 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-dichloroindolepheno.

### **Reagents and Equipment Required but Not Provided:**

Spectrophotometer, water bath, desk centrifuge, transferpeltor, 1ml glass cuvette, mortar/ homogenizer, acetone, ice and distilled water.

#### **1. 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°C for 10min. Discard the precipitate and transfer supernatant to another tube, centrifuge at 11000g and 4°C 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;
- 4) Add 400 μL 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 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 according to 1:1 before use. Prepare when use. Prepared when the solution will be used.
- 2) Preheat reagent1 at 37°C (mammal cell) or 25°C(other species) for 15 min.
- 3) Add the following reagents in 1ml glass cuvette:

Reagent name (uL)	Test tube (A1)
Sample	50
Reagent 1	750
Working solution	100
Reagent 4	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°C(mammal) or 25°C(other species) water bath for 2 min, then take cuvette quickly, dry and detect absorbance at 2 min (A2),  $\Delta A = A1 - A2$

### 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.

$$\text{Complex II Activity (U/mg prot)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (V_s \times C_{pr}) \div T = 476.2 \times \Delta A \div C_{pr}$$

$\epsilon$ : 2, 6-dichlorindolepheno molar extinction coefficient,  $2.1 \times 10^4 \text{L/mol/cm}$ ;

$d$ : light path of cuvette, 1cm;

$V_{rv}$ : total reaction volume, 1mL;

$V_s$ : sample volume (mL), 0.05 mL;

$C_{pr}$ : sample protein concentration (mg/mL);

$T$ : reaction time (min), 2 min;

### 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  $\Delta A > 0.4$ , multiply dilute times in the formula. Increase sample volume if  $\Delta A$  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 50 tube reactions.
5. Attachment: Sample weight (50T/24S)
  - 1) 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.

$$\text{Complex II Activity(U/g)} = [\Delta A1 \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (W \div V_e \times V_s) \div T = 476.2 \times \Delta A1 \div W$$

$\Delta A1$ : supernatant absorbance;

$V_{rv}$ : total reaction volume, 1mL;

$\epsilon$ : 2, 6-dichlorindolepheno molar extinction coefficient,  $2.1 \times 10^4$  L/mol/cm;

$d$ : light path of cuvette, 1cm;

$V_e$ : extract solution volume, 1mL;

$V_s$ : sample volume (mL), 0.05 mL;

$T$ : reaction time (min), 2 min;

$W$ : sample weight, g.

2) 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.

$$\text{Complex II Activity(U/g)} = [\Delta A2 \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (W \div V_e \times V_s) \div T = 190.5 \times \Delta A2 \div W$$

$\Delta A2$ : sediment absorbance;

$V_{rv}$ : total reaction volume, 1mL;

$\epsilon$ : 2, 6-dichlorindolepheno molar extinction coefficient,  $2.1 \times 10^4$  L/mol/cm;

$d$ : light path of cuvette, 1cm;

$V_e$ : sediment resuspended volume, 0.4 mL;

$V_s$ : sample volume (mL), 0.05 mL;

$T$ : reaction time (min), 2 min;

$W$ : sample weight, g.

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

$$\text{Complex II(U/g)} = 476.2 \times \Delta A1 \div W + 190.5 \times \Delta A2 \div W.$$

### 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.  $\Delta A1 = A1 - A2 = 1.134 - 1.054 = 0.08$  in the supernatant, and  $\Delta A2 = A1 - A2 = 1.371 - 1.347 = 0.024$  in the precipitation.

The activity of complex II in the supernatant (U/g mass) =  $476.2 \times \Delta A1 \div W = 476.2 \times 0.08 \div 0.1 = 380.96$  U/g mass

The activity of complex II in the precipitation (U/g mass) =  $190.5 \times \Delta A2 \div W = 190.5 \times 0.024 \div 0.1 = 45.72$  U/g mass

Complex II (U/g mass) =  $476.2 \times \Delta A1 \div W + 190.5 \times \Delta A2 \div W = 476.2 \times 0.08 \div 0.1 + 190.5 \times 0.024 \div 0.1 = 426.68$  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] Mühlhling J, Tiefenbach M, López-Barneo J, et al. Mitochondrial complex II participates in normoxic and hypoxic regulation of  $\alpha$ -keto acids in the murine heart[J]. *Journal of molecular and cellular cardiology*, 2010, 49(6): 950-961.

#### **Related Products:**

NA0828/NA0586 Electron Transport Chain Complex I Activity Assay Kit

NA0638/NA0396 Electron transport chain Complex III Activity Assay Kit

NA0800/NA0559 Electron transport chain Complex IV Activity Assay Kit

NA0755/NA0513 Electron transport chain Complex V Activity Assay Kit