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

Mitochondrial Respiratory(Electron transport) Chain Complex Ⅲ Activity Assay Kit

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

Operation Equipment: spectrophotometer/ microplate reader

Cat No: AK0365-100T-48S

Components:

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

Reagent I: Liquid 10ml×2. Store at 2-8℃.

Reagent II: Powder×2. Store at -20℃.

Reagent III: Liquid 1.5mL×1. Store at 2-8℃.

Working solution: Before use, transfer one Reagent II to one Reagent I for mixing and dissolution (about 62T). It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.

Product Description:

Mitochondrial complex III (EC 1.10.2.2), also known as CoQ-cytochrome C reductase, is widely found in mitochondria of animals, plants, microorganisms and cultured cells. It is a common component of the main pathway and branch of the mitochondrial respiratory electron transport chain. Mitochondrial complex III is responsible for transferring hydrogen from reduced CoQ to cytochrome C and then produce reduced cytochrome C.

Unlike oxidized cytochrome C, reduced cytochrome C has a characteristic absorption at 550 nm, so the rate of increase in light absorption at 550 nm can reflect mitochondrial complex III enzyme activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ Microplate reader, water bath, desk centrifuge, transferpettor, micro glass cuvette/ 96-well flat-bottom plate(non-polystyrene material), mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedures:

I. Complex III 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 III leaking from mitochondria, this step can show the effect of mitochondrial extraction;
4) Add 200ul of extraction solution to sediment, splitting with ultrasonication (power 200W, work time 5s，interval 10s，repeat 15 times), used to detect Complex III activity and protein content.

II. Determining step

1) Preheat spectrophotometer/microplate reader for 30min, adjust the wavelength to 550 nm, set the spectrophotometer counter to zero with distilled water.
2) Add the following reagents in micro glass cuvette/ 96-well flat-bottom plate:

Reagent (μL)	Test tube (At)	Control tube (Ac)
Working solution	160	160
Reagent III	20	-
Accurately incubate for 2 min at 37 °C (mammal) or 25 °C (other species), then add separately as follows		
Sample	20	20
Distilled water	-	20
Mix thoroughly, detect absorbance at 10s (At1 and Ac1). Put micro glass cuvette/ 96-well flat-bottom plate and react solution together in 37℃(mammal) or 25℃ (other species) water bath for 2 min, then take it out quickly, dry and detect absorbance at 2 min (At2 and Ac2), ΔA= (At2-At1) - (Ac2-Ac1). Each test tube needs one control tube.		

III. Calculation:

1. Micro cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1nmol of reduced cytochrome C per mg of tissue protein in every minute.

Complex III Activity (U/mg prot) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (Vs \times Cpr) \div T = 262 \times \Delta A \div Cpr$

ε: Reduced cytochrome C molar extinction coefficient, 1.91×10^4L/mol/cm;

d: light path of cuvette, 1cm;

Vrv: total reaction volume,0.0002L;

Vs: sample volume (mL), 0.02 mL;

Cpr: sample protein concentration (mg/mL);

T: reaction time (min), 2 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 for calculation.

Note:

1. Try to keep the temperature of the reaction solution in micro glass cuvette/ 96-well flat-bottom plate at 37℃ or 25℃. After recording the initial absorbance A1, put it together with the reaction solution into a water bath of 37℃ (mammal) or 25℃ (other species) to react accurately for 2 minutes, then take it out quickly and dry it, and record the absorbance at 2 minutes.
2. When the absorbance value is greater than 1, it is recommended to dilute the sample with extraction solution and then determine it. Pay attention to multiply the dilution multiple in the calculation formula.
3. Detect sample protein concentrate by yourself.
4. 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 (measured separately).

5. 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.
6. It's enough for 100 tube reactions.
7. Attachment: Sample weight (100T/24S)

A. Supernatant:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1nmol of reduced cytochrome C in 1min every gram of tissue weight.

Complex III Activity (U/g)=[ΔA1×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =262×ΔA1÷W

ΔA1: supernatant absorbance;

Vrv: total reaction volume,0.0002L;

ε: Reduced cytochrome C molar extinction coefficient, 1.91×10^4L/mol/cm;

d: light path of cuvette, 1cm;

Ve: extract solution volume,1mL;

Vs: sample volume (mL), 0.02mL;

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 reduced cytochrome C in 1min every gram of tissue weight.

Complex III Activity (U/g)= [ΔA2×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =52×ΔA2÷W

ΔA2: sediment absorbance;

Vrv: total reaction volume,0.0002 L;

ε: Reduced cytochrome C molar extinction coefficient, 1.91×10^4L/mol/cm;

d: light path of cuvette, 1cm;

Ve: extract solution volume,0.2mL;

Vs: sample volume (mL), 0.102mL;

T: reaction time (min), 2 min;

W: sample weight, g.

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

Complex III Activity(U/g) =262×ΔA1÷W +52×ΔA2÷W.

Experimental Example:

1. Take 0.1g rabbit kidney for sample treatment, dilute twice the precipitate after re dissolving, and then operate according to the determination steps. Calculate according to the sample protein concentration:

   $\Delta A = (A_{2T} - A_{1T}) - (A_{2C} - A_{1C}) = (0.9921-0.9077) - (0.9664-0.9419) = 0.0599$

   Complex III Activity(U/mg prot) = 262×0.0599÷2.56×2 (dilution ratio) = 12.26 U/mg prot.

References：

[1] Luo C, Long J, Liu J. An improved spectrophotometric method for a more specific and accurate assay of mitochondrial complex III activity[J]. Clinica Chimica Acta, 2008, 395(1-2): 38-41.