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: NA0396

Size:100T/48S

Components:

Extract solution: 80 ml×1. Storage at 4℃.

Reagent 1: 20 ml×1. Storage at 4℃.

Reagent 2: Powder×1. Storage at -20℃.

Reagent 3: 2.5 mL×1. Storage at 4℃.

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, ice and distilled water.

Procedures:

1. 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 10 min. 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 shows the effect of mitochondrial extraction;
4) Add 200ul of extraction solution to sediment, splitting with ultrasonication (power 20%, work time 5s，interval 10s，repeat 15 times), used to detect Complex III activity and protein content.

2. Determining step

1) Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 550 nm, set the counter to zero with distilled water.

2) Making working solution: Transfer one bottle of reagent2 to one bottle of reagent1 to dissolve thoroughly before use, and unused reagent at 4 °C for one week;

3) Add the following reagents in 1ml cuvette:

Reagent name (uL)	Test tube (At)	Control tube (Ac)
Working solution	160	160
Reagent 3	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
Add the above reagent to the cuvette, mix thoroughly, detect absorbance at 10s (At1 and Ac1). 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 (At2 and Ac2), ΔA= (At2-At1) - (Ac2-Ac1)		

3. 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) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (Vs \times Cpr) \div T = 261 \times \Delta A \div Cpr$

ε: Reduced cytochrome C molar extinction coefficient, 19100L/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;

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 the cuvette at 37℃ or 25℃. After recording the initial absorbance A1, put the cuvette together with the reaction solution into a water bath of 37℃ (mammal) or 25℃ (other species) to react accurately for 2 minutes, then take out the cuvette 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, you can use Solarbio (PC0020 BCA Protein Assay Kit).
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)

1) 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,0.001L;
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), 0.1 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 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,0.001 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), 0.1mL;
T: reaction time (min), 2 min;
W: sample weight, g.

3) Total activity is the sum of Complex III activity in supernatant and sediment.
Complex III(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: ΔA = (A_{2T} - A_{1T}) - (A_{2C} - A_{1C}) = (0.9921-0.9077) - (0.9664-0.9419) = 0.0599

Complex III (U/mg prot) = 262×ΔA÷ Cpr ×2 (dilution ratio) = 262 × 0.0599÷2.56 ×2 (dilution ratio) =

12.26 U/mg prot.

Recent Product Citations：

[1] Ming Song,Fangfang Chen,Yihui Li,et al. Trimetazidine restores the positive adaptation to exercise training by mitigating statin-induced skeletal muscle injury. Journal of Cachexia, Sarcopenia and Muscle. November 2017;(IF10.754)

[2] Liuqin He,Haiwen Zhang,Xihong Zhou. Weanling Offspring of Dams Maintained on Serine-Deficient Diet Are Vulnerable to Oxidative Stress. Oxidative Medicine and Cellular Longevity. September 2018;(IF4.868)

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

[4] 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.

[5] 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] 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.

Related Products：

NA0828/NA0586 Electron Transport Chain Complex I Activity Assay Kit
NA0639/NA0397 Electron transport chain Complex II Activity Assay Kit
NA0800/NA0559 Electron transport chain Complex IV Activity Assay Kit
NA0755/NA0513 Electron transport chain Complex V Activity Assay Kit