（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K150-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（590-610 nm）

Detection range：0.54-17.66 U/L

Elabscience® Mitochondrial Complex II Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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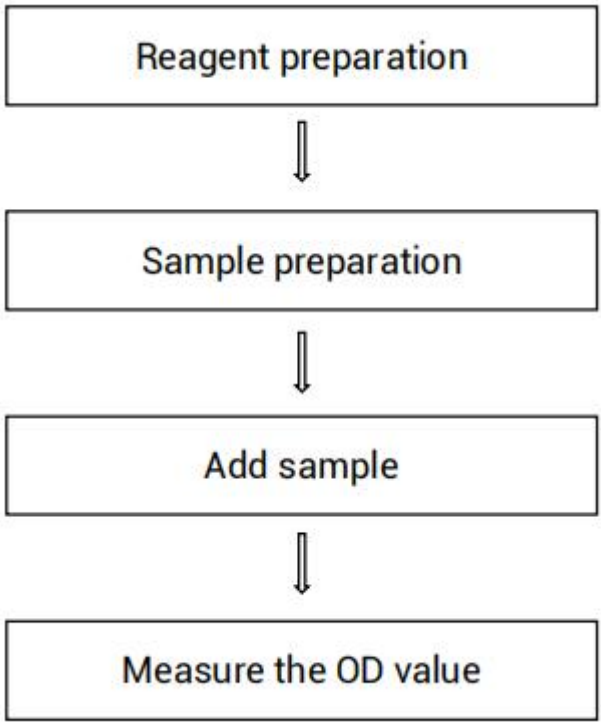
Assay summary



Intended use

This kit can measure mitochondrial complex II activity in animal tissue samples.

Detection principle

Mitochondrial complex II, also known as succinate-coenzyme Q reductase, is widely found in the mitochondria of animals, plants, microorganisms and cultured cells. It catalyzes the oxidation of succinic acid in the TCA cycle to fumaric acid, which converts the ubiquinone to a reduced form through the basic unit structures of the complex such as iron-sulfur proteins. Coenzyme Q, the catalytic product of mitochondrial complex II, can further reduce 2, 6-dichloroindoxol, which has a characteristic absorption peak at 600 nm. Therefore, the activity of mitochondrial complex II can be quantified by measure the change OD value at 600 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Extraction Solution B	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months
Reagent 3	Inhibitor	0.8 mL × 1 vial	0.8 mL × 2 vials	-20°C, 12 months, shading light
Reagent 4	Buffer Solution	10 mL×1 vial	20 mL×1 vial	-20°C, 12 months
Reagent 5	Substrate A	0.8 mL×1 vial	1.6 mL×1 vial	-20°C, 12 months, shading light
Reagent 6	Substrate B	1.5 mL×1 vial	1.5 mL×2 vials	-20°C, 12 months, shading light
Reagent 7	Substrate C	0.6 mL×1 vial	1.2 mL×1 vial	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (590-610 nm, optimum wavelength: 600 nm), Centrifuge

Reagents:

Normal saline (0.9% NaCl), PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of reaction working solution：

For each well, prepare 190 μL of reaction working solution (mix well 150 μL of buffer solution, 10 μL of substrate A, 20 μL of substrate B and 10 μL of substrate C). The reaction working solution should be prepared on spot. Store for 1 day protected from light.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 100 mg tissue in 900 μL extraction solution A with a dounce homogenizer at 4°C.

④ Centrifuge at 600×g for 5 min, discard the precipitate and take the supernatant.

⑤ Then centrifuge at 15000×g for 10 min at 4°C, discard the supernatant and take the precipitate.

⑥ The precipitate was mixed with 200 μL of extraction solution B and 10 μL of inhibitor, sonicated for 1 min at 4°C, centrifuged at 15000×g at 4°C for 10 min. Then take the supernatant for detection.

⑦ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1

Note: The diluent is extraction solution B. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① When adding the reaction working solution, adhere to the wall and add slowly to avoid bubbles.

② Use fresh samples for detection, processed samples should be tested the same day.

③ It is recommended that the number of samples for an experiment be controlled within 10 samples.

Operating steps

① Blank well: Add 190 μL of reaction working solution to each well.
Sample well: Add 190 μL of reaction working solution to each well.

② Incubate at 37°C for 3 min.

③ Blank well: Add 20 μL of extraction solution B to blank well.
Sample well: Add 20 μL of sample to sample well.

④ Mix fully with microplate reader for 3 s and measure the OD value of each well at 600 nm with microplate reader, recorded as A_1.

⑤ Incubate at 37°C for 3 min, measure the OD value of each well at 600 nm with microplate reader, recorded as A_2, $\Delta A = A_1 - A_2$.

Note: When adding the reaction working solution, adhere to the wall and add slowly to avoid bubbles.

Calculation

The sample:

For tissue samples:

Definition: The amount of mitochondrial complex II in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol product at room temperature is defined as 1 unit.

$$\text{mitochondrial complex II activity (U/gprot)} = \frac{(\Delta A_{sample} - \Delta A_{blank}) \times V_{total} \times f}{(V_{sample} \times 21.8^{*} \times T \times C_{pr})} \times 1000^{*}$$

[Note]

ΔA_{sample}: The change OD value of sample (A_1 - A_2).

ΔA_{blank}: The change OD value of blank (A_1 - A_2).

f: Dilution factor of sample before test.

V_{total}: The volume of the reaction system, 0.21 mL.

V_{sample}: The volume of the sample, 0.02 mL.

21.8*: Molar absorption coefficient.

C_{pr}: The concentration of protein in sample, gprot/L.

T: The time of reaction, 3 min.

1000*: 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat heart tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.35	12.60	15.50
%CV	4.3	3.8	3.9

Inter-assay Precision

Three rat heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.35	12.60	15.50
%CV	4.9	5.2	4.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	4.5	9.8	14.5
Observed Conc. (μmol/L)	4.5	9.6	13.3
Recovery rate (%)	101	98	92

Sensitivity

The analytical sensitivity of the assay is 0.54 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

Example analysis：

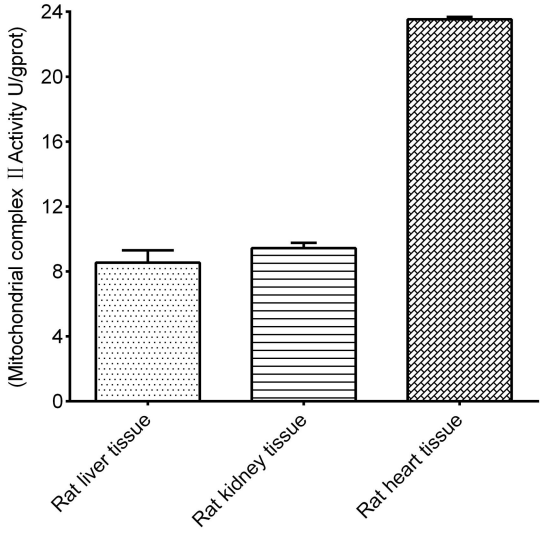
For rat heart tissue, take 20 μL of 10% rat heart tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

the OD value of the blank A_1 is 0.979, the OD value of the blank A_2 is 0.972, the OD value of the sample A_1 is 0.671, the OD value of the sample A_2 is 0.310, the concentration of protein in sample is 2.07 gprot/L, and the calculation result is:

$$\text{mitochondrial complex II activity (U/gprot)} = \frac{(0.671 - 0.310) - (0.979 - 0.972) \times 0.21}{0.02 \times 21.8 \times 3 \times 2.07} \times 1000$$

$$= 27.61 \text{ U/gprot}$$

Detect 10% rat liver tissue homogenate (the concentration of protein is 10.29 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein is 10.09 gprot/L), 10% rat heart tissue homogenate (the concentration of protein is 2.07 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Tian Y , Hong X , Xie Y ,et al.17β-Estradiol (E 2) Upregulates the ERα/SIRT1/PGC-1α Signaling Pathway and Protects Mitochondrial Function to Prevent Bilateral Oophorectomy (OVX)-Induced Nonalcoholic Fatty Liver Disease (NAFLD)[J].Antioxidants, 2023, 12(12).DOI:10.3390/antiox12122100.
2. Zhang Y , Luo C , Huang P ,et al.Luteolin alleviates muscle atrophy, mitochondrial dysfunction and abnormal FNDC5 expression in high fat diet-induced obese rats and palmitic acid-treated C2C12 myotubes[J].The Journal of Nutritional Biochemistry, 2025, 135.DOI:10.1016/j.jnutbio.2024.109780.
3. Xiao P , Wu S , Wang Z ,et al.Biotoxicity of paraquat to lung cells mediated by endoplasmic reticulum-mitochondria interaction[J].Journal of Molecular Histology, 2024, 55(6):1063-1077.DOI:10.1007/s10735-024-10249-7.
4. Chen L , Chen S , Bai Y ,et al.Electroacupuncture improves cognitive impairment after ischemic stroke based on regulation of mitochondrial dynamics through SIRT1/ PGC-1α pathway[J].Brain research, 2024:1844.DOI:10.1016/j.brainres.2024.149139.
5. Fu R , Guo X , Pan Z ,et al.Molecular mechanisms of AMPK/YAP/NLRP3 signaling pathway affecting the occurrence and development of ankylosing spondylitis[J].Journal of orthopaedic surgery and research, 18(1):831.DOI:10.1186/s13018-023-04200-x.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.