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

Catalog No：E-BC-K151-M

Specification：48T(23 samples)/96T(47 samples)

Measuring instrument：Microplate reader（540-560 nm）

Detection range：4.45–106.8 U/L

Elabscience® Mitochondrial Complex Ⅲ (Coenzyme Q-Cytochrome C Reductase) 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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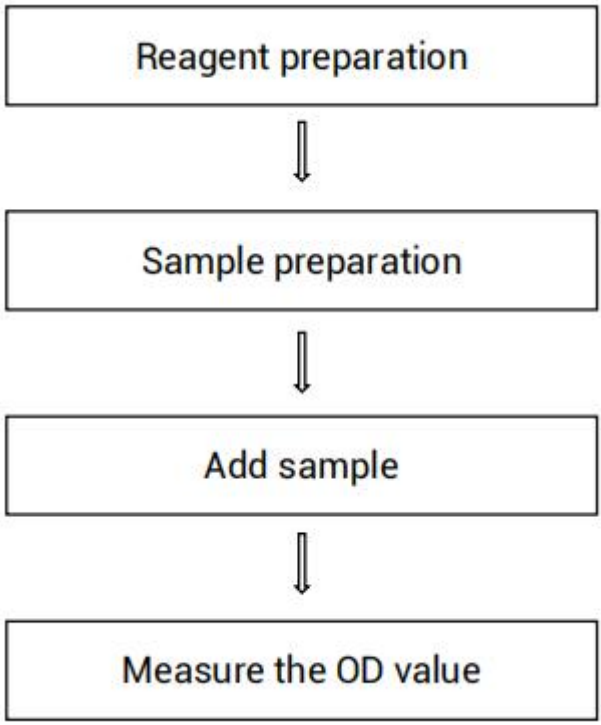
Assay summary



Intended use

This kit can measure mitochondrial complex III (Coenzyme Q-Cytochrome C Reductase) activity in animal tissue sample.

Detection principle

As an organelle, mitochondria is the "power factory" in cells and the main site of aerobic respiration of cells. Its function is to convert energy through oxidative phosphorylation to provide energy for cellular activities. The oxidation process is carried out by four respiratory chain membrane protein complexes (complexes I, II, III and IV) on the inner mitochondrial membrane. Mitochondrial complex III, also known as cytochrome c reductase complex, its main function is to oxidize the reduced coenzyme Q10 formed by mitochondrial complexes I and II to oxidative coenzyme Q10. In this process, the OD value increased at 550 nm. Therefore, the activity of mitochondrial complex III can be quantified by measure the change OD value at 550 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	55 mL × 1 vial	55 mL × 2 vials	-20°C, 12 months
Reagent 2	Extraction Solution B	13 mL × 1 vial	26 mL × 1 vial	-20°C, 12 months
Reagent 3	Extraction Solution C	1.2 mL × 1 vial	1.2 mL × 2 vials	-20°C, 12 months, shading light
Reagent 4	Substrate A	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Diluent	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
Reagent 6	Substrate B	0.8 mL × 1 vial	1.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	Stabilizer	Powder × 3 vials	Powder × 6 vials	-20°C, 12 months, shading light
Reagent 8	Buffer Solution	13 mL × 1 vial	26 mL × 1 vial	-20°C, 12 months
Reagent 9	Inhibitor Controlled Solution	1.5 mL × 1 vial	3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 10	Inhibitor	1.5 mL × 1 vial	3 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layou t 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:

Centrifuge, Microplate reader (540-560 nm, optimum wavelength: 550 nm)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate B before use：
Place the substrate B at 37°C for 10 min before use, and mix the solution until its clarified for use. Aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of stabilizer working solution：
Dissolve one vial of stabilizer with 2 mL of diluent, mix well. Store on ice protected from light and be used up within 6 hours.

④ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 15 μL of reaction working solution (mix well 5 μL of stabilizer working solution and 10 μL of substrate B). Stand the prepared solution at room temperature protected from light for 3 min, then use immediately, the reaction working solution is a white suspension. And the reaction working solution should be used within 30 min.

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, and 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 extraction solution C, sonicated for 1 min, centrifuged at 11000×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	1-2
10% Rat kidney tissue homogenate	1-2
10% Rat brain tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1-2
10% Mouse kidney tissue homogenate	1-2
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen 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 The mitochondrial samples should be detected within 4 h as far as possible. If the samples are placed for a long time, the specific enzyme activity will be reduced, and the results of the sample determination will be low.

② The detection is started at about 10 s after adding substrate A, and it's better to measure no more than 4 samples at same time.

③ The change OD value of blank well should be within ± 0.005, indicating that the reagents are available. If it exceeds this range, it is necessary to check whether substrate B is clear and extend the incubation time.

Operating steps

1. Sample pretreatment

Total enzyme activity sample: Mix the sample and inhibitor controlled solution at the volume of 20 μL: 20 μL fully. Stand the prepared solution at room temperature protected from light for 5 min for use.

Non-specific enzyme activity sample: Mix the sample and inhibitor at the volume of 20 μL: 20 μL fully. Stand the prepared solution at room temperature protected from light for 5 min for use.

2. The measurement of samples

① Blank well: Add 10 μL of reaction working solution to the corresponding wells.

Total enzyme activity sample well: Add 10 μL of reaction working solution to the corresponding wells.

Non-specific enzyme activity sample: Add 10 μL of reaction working solution to the corresponding wells.

② Add 90 μL of buffer solution to each well.

③ Blank well: Add 20 μL of extraction solution B to the corresponding wells.

Total enzyme activity sample well: Add 20 μL of total enzyme activity sample to the corresponding wells.

Non-specific enzyme activity sample well: Add 20 μL of non-specific enzyme activity sample to the corresponding wells.

④ Add 40 μL of substrate A to each well. Mix fully with microplate reader for 5 s. (Because of the rapid enzymatic reaction, it is recommended to add substrate A using a multi-pipe pipettor, and it's better to measure no more than 4 samples at same time when using ordinary pipettor)

⑤ Measure the OD value of each well at 10 s and 70 s respectively at 550 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_2 - A_1$.

Calculation

For tissue sample:

Definition: The amount of mitochondrial complex III in 1 g tissue mitochondria protein per 1 minute that reduce 1 μmol cytochrome c at room temperature is defined as 1 unit.

$$\text{mitochondrial complex III activity (U/gprot)} = \frac{(\Delta A_{Total} - \Delta A_{Non\text{-}specific}) \times V_1 \times f}{V_2 \times (\varepsilon \times d) \times T} \div C_{pr}$$

[Note]

ΔA_{Total}: The change OD value of total enzyme activity sample well ($A_2 - A_1$).

$\Delta A_{Non\text{-}specific}$: The change OD value of non-specific enzyme activity sample well ($A_2 - A_1$).

V_1: The volume of the reaction system, 0.16 mL.

V_2: The volume of the sample, 0.02 mL.

ε: The molar extinction coefficient of cytochrome c at 550 nm, 0.0191 L/μmol/cm

d: Optical path, 0.5 cm

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat brain 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)	10.50	56.00	88.50
%CV	5.2	4.9	4.9

Inter-assay Precision

Three rat brain 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)	10.50	56.00	88.50
%CV	9.8	10.2	10.0

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	22.5	76.3	92.5
Observed Conc. (U/L)	22.7	75.5	92.5
Recovery rate (%)	101	99	100

Sensitivity

The analytical sensitivity of the assay is 4.45 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

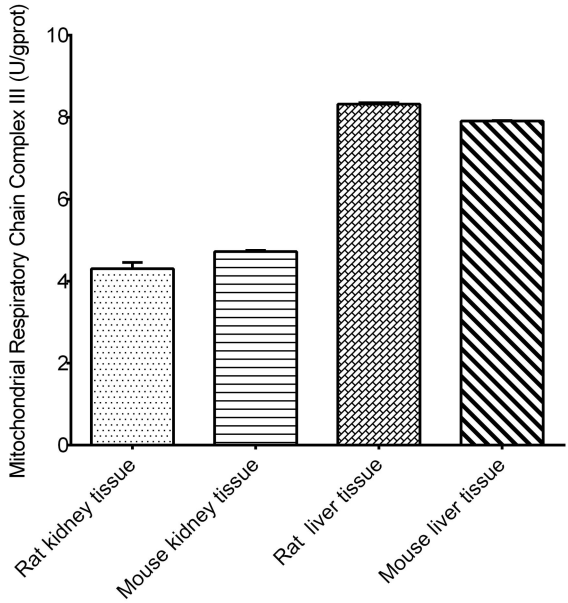
Example analysis：

For 10% rat kidney tissue mitochondria supernatant, dilute for 2 times, carry the assay according to the operation steps. The results are as follows:

The A_1 of the blank well is 0.198, the A_2 of the blank well is 0.199, ΔA = 0.001, indicating that the reagents are available. The A_1 of total enzyme activity sample well is 0.239, the A_2 of total enzyme activity sample well is 0.306, $\triangle A_{Total}$ = 0.067. The A_1 of non-specific enzyme activity sample well is 0.235, the A_2 of non-specific enzyme activity sample well is 0.289, $\Delta A_{Non\text{-}specific}$ = 0.054., the concentration of mitochondria protein in sample is 5.24 gprot/L, and the calculation result is:

$$\text{mitochondrial complex III activity (U/gprot)} = \frac{(0.067 - 0.054) \times 0.16 \times 2}{0.02 \times 0.01917 \times 0.5 \times 1} \div 5.24 = 4.16 \text{ U/gprot}$$

Detect 10% rat kidney tissue homogenate (the concentration of mitochondria protein is 5.24 gprot/L, dilute for 2 times), 10% mouse kidney tissue homogenate (the concentration of mitochondria protein is 6.76 gprot/L), 10% rat liver tissue homogenate (the concentration of mitochondria protein is 4.05 gprot/L, dilute for 4 times) and 10% mouse liver tissue homogenate (the concentration of protein is 5.12 gprot/L) according to the protocol, the result is as follows：


Mitochondrial Respiratory Chain Complex III (U/gprot)
10
8
6
4
2
0
Rat kidney tissue
Mouse kidney tissue
Rat liver tissue
Mouse liver tissue

Appendix III Publications

1. 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.
2. 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.

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.