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

Catalog No：E-BC-K152-M

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

Measuring instrument：Microplate reader（540-560 nm）

Detection range：3.0–88.6 U/L

Elabscience® Mitochondrial Complex IV （Cytochrome C Oxidase）Activity Assay Kit

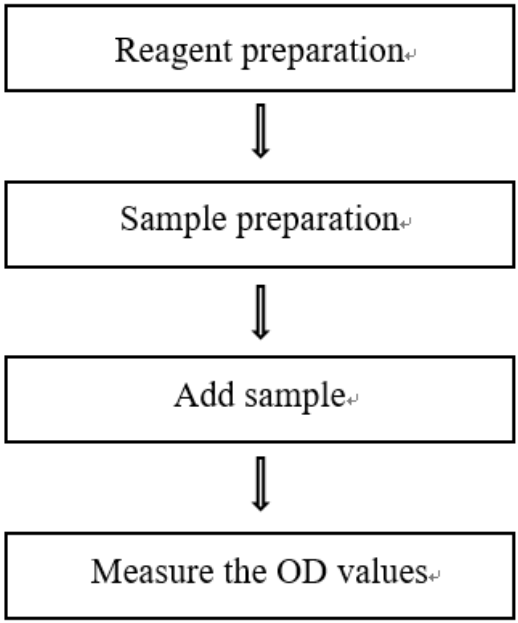
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can measure mitochondrial complex IV（cytochrome C oxidase）activity in animal tissue samples.

Detection principle

Mitochondrial complex IV, also known as cytochrome C oxidase, is one of the major enzymes in the mitochondrial respiratory chain. It oxidizes the reduced cytochrome C converted from mitochondrial complex III to oxidized cytochrome C and consumes oxygen to generate water. Mitochondrial complex IV can catalyze the oxidation of reduced cytochrome C to oxidized cytochrome C, which has an absorption wavelength at 550 nm. Therefore, the activity of mitochondrial complex IV 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	Extracting Solution A	50 mL ×1 vial	50 mL ×2 vials	-20℃, 12 months
Reagent 2	Extracting Solution B	25 mL ×1 vial	50 mL ×1 vial	-20℃, 12 months
Reagent 3	Inhibitor	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 4	Substrate	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 5	Stabilizer	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 6	Buffer Solution	13 mL×1 vial	26 mL×1 vial	-20℃, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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

Reagents:

Anhydrous ethanol, PBS(0.01 M，pH 7.4)

Reagent preparation

① Keep inhibitor, substrate and stabilizer on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of inhibitor working solution：

Dissolve one vial of inhibitor with 1 mL of anhydrous ethanol, mix well. Store

at -20 ℃ for 1 month protected from light.

③ The preparation of substrate working solution：

Dissolve one vial of substrate with 4 mL of buffer solution, mix well. Aliquoted storage at -20 ℃ for 1 month protected from light, and avoid repeated freeze/thaw cycles is advised.

④ The preparation of stabilizer working solution：

Dissolve one vial of stabilizer with 200 μL of buffer solution, mix well. Storage at -20 ℃ for 1 month protected from light, and avoid repeated freeze/thaw cycles is advised.

⑤ The preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 103 μL of reaction working solution (mix well 100 μL of substrate working solution and 3 μL of stabilizer working solution). The reaction working solution should be prepared on spot. The reaction working solution should be placed at room temperature protected from light for 10 min before use.

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 ℃.

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

⑤ Then centrifuge at 11000×g for 10 min at 4 ℃, discard the supernatant and take the precipitate.

⑥ The precipitate was mixed with 200 μL of extraction solution B and 10 μL of inhibitor working solution, sonicated for 1 min, centrifuged at 11000×g at 4 ℃ 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 kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen tissue homogenate	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① It is recommended that the number of samples for an experiment be controlled within 5 samples.

② The average ΔA value of blank well should be within ±0.005.

Operating steps

① Blank well: Add 30 μL of extraction solution B to blank well.
Sample well: Add 30 μL of sample to sample well.

② Add 120 μL of buffer solution to each well and mix fully with microplate reader for 3 s.

③ Add 70 μL of reaction working solution to each well.

④ Measure the OD value of each well at 550 nm with microplate reader at 10s and 70s, respectively recorded as A_1 and A_2, $\Delta A = A_1 - A_2$.

Note: The average ΔA value of blank well should be within ±0.005.

Calculation

For tissue sample:

Definition: The amount of mitochondrial complex IV in 1 g tissue mitochondrial protein per 1 minute that oxidize 1 μmol of cytochrome C at room temperature is defined as 1 unit.

$$\text{mitochondrial complex IV activity (U/gprot)} = \frac{\Delta A_{550} \times V_1}{V_2 \times (\varepsilon \times d) \times T} \div C_{pr} \times f$$

[Note]

ΔA_{550}: $\Delta A_{sample} - \Delta A_{blank.}$

V_1: The volume of the reaction system, 0.22 mL.

V_2: The volume of the sample, 0.03 mL.

ε: Molar absorption coefficient, 0.0191 L/μmol/cm.

d: Optical path, 0.65 cm

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

C_{pr}: The concentration of 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 (μmol/L)	8.50	34.60	70.50
%CV	5.1	5.0	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 (μmol/L)	8.50	34.60	70.50
%CV	9.8	9.1	9.6

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 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	18.5	42.7	65.1
Observed Conc. (μmol/L)	18.7	44.8	69.0
Recovery rate (%)	101	105	106

Sensitivity

The analytical sensitivity of the assay is 3.0 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 II Example Analysis

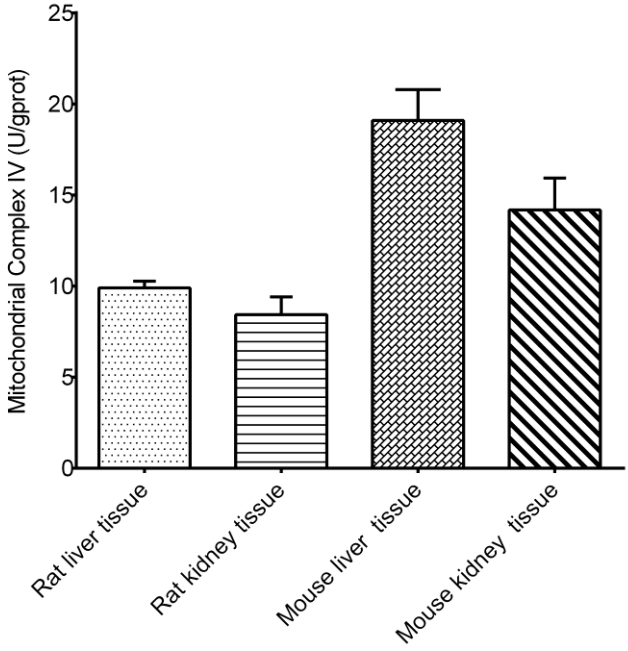
Example analysis:

For rat liver tissue, take 30 μL of 10% rat liver tissue mitochondrial supernatant, and carry the assay according to the operation steps. The results are as follows:

the OD value of the sample A_1 is 0.458, the OD value of the sample A_2 is 0.402, the OD value of the blank A_1 is 0.394, the OD value of the blank A_2 is 0.393, the concentration of protein in sample is3.32 gprot/L, and the calculation result is:

$$\text{mitochondrial complex IV (U/gprot)} = \frac{((0.458 - 0.402) - (0.394-0.393)) \times 0.22}{0.03 \times 0.0191 \times 0.65 \times 1} \div 3.32 = 9.79 \text{ U/gprot}$$

Detect 10% rat liver tissue homogenate (the concentration of mitochondrial protein is 3.32 gprot/L), 10% rat kidney tissue homogenate (the concentration of mitochondrial protein is 6.49 gprot/L), 10% mouse liver tissue homogenate (the concentration of mitochondrial protein is 3.17 gprot/L) and 10% mouse kidney tissue homogenate (the concentration of mitochondrial protein is 2.37 gprot/L) according to the protocol, the result is as follows:


Mitochondrial Complex IV (U/gprot)
25
20
15
10
5
0
Rat liver tissue
Rat kidney tissue
Mouse liver tissue
Mouse kidney tissue

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.