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

Catalog No：E-BC-K149-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（340 nm）

Detection range：4.33-224.2 U/L

Elabscience® Mitochondrial Complex I (NADH-CoQ 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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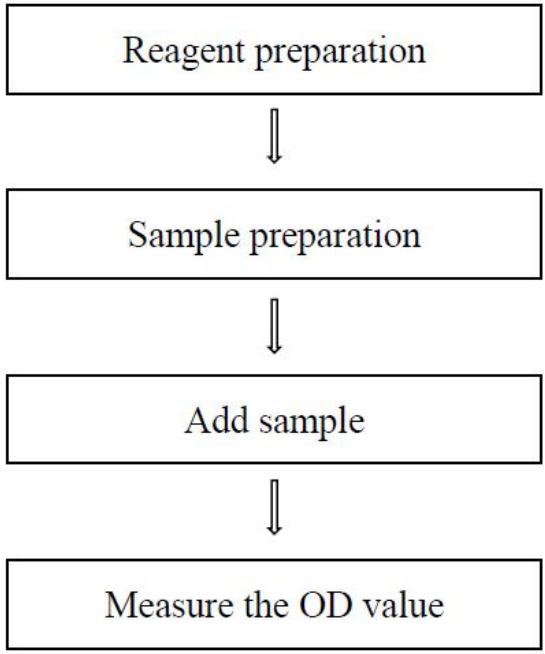
Assay summary



Intended use

This kit can measure mitochondrial complex I (NADH-CoQ Reductase) activity in animal tissue sample.

Detection principle

Mitochondrial complex I catalyzes the reaction of NADH with ubiquinone substrate to generate NAD+ and reduced ubiquinone. The activity of NADH can be reflected by measuring the absorbance decline rate at 340 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	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 3	Protease Inhibitor	0.4 mL × 1 vial	0.4 mL × 2 vials	-20°C, 12 months, shading light
Reagent 4	Buffer Solution	15 mL × 1 vial	15 mL × 2 vials	-20°C, 12 months, shading light
Reagent 5	Substrate A	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 6	Substrate B	Powder × 1 vial	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 7	Inhibitor	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, shading light
Reagent 8	Negative Reagent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, shading light
	UV 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:

Centrifuge, 37°C incubator, Microplate reader (340 nm)

Reagents:

Anhydrous ethanol (AR)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A solution：
Dissolve one vial of substrate A with 150 μL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

③ The preparation of substrate A working solution：
Before testing, please prepare sufficient working solution according to the sample wells. For example, prepare 1012 μL of substrate A working solution (mix well 12 μL of substrate A solution and 1000 μL of buffer solution). Store at 2-8°C for 12 h protected from light.

④ The preparation of substrate B working solution：
Dissolve one vial of substrate B with 4 mL of anhydrous ethanol and shake until it turned yellowish clear liquid. Store at 2-8°C for 48 h protected from light. Aliquoted storage at -20°C for 7 days protected from light.

⑤ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare250 μL of reaction working solution (mix well 5 μL of substrate B working solution and 245 μL of substrate A working solution). The reaction working solution should be prepared on spot protected from light, keep on the ice box during use within 1 h.

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 and 10μL protease inhibitor with a dounce homogenizer at 4°C.

④ Centrifuge at 600×g at 4°C 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 2 μL of protease inhibitor, sonicated for 1 min, 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 muscle tissue homogenate	1-2
10% Rat lung tissue homogenate	4-8
10% Mouse liver tissue homogenate	1-2
10% Mouse heart tissue homogenate	4-8
10% Rat heart tissue homogenate	1-2
10% Rat liver tissue homogenate	1-2
10% Rat kidney tissue homogenate	2-4
10% Porcine heart tissue homogenate	1-2

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 During reagent preparation, it is necessary to ensure that the prepared substrate B working solution is completely dissolved. It is recommended to extend the time of oscillation, transfer the reagent in a EP tube to check whether the reagent is completely dissolved to clear.

② During sample measurement, if the OD value decreases by more than 0.3 within 3 min, the sample should be diluted to ensure that the sample measurement is within the interval of uniform reaction speed. If necessary, the OD value can be measured every minute to observe its dynamics changes.

③ It is recommended to use fresh sample for detection.

④ It's better to measure no more than 8 sample wells at same time.

Operating steps

① Control well: Add 20 μL of sample to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.

② Control well: Add 20 μL of negative reagent to the corresponding wells.
Sample well: Add 20 μL of inhibitor to the corresponding wells.

③ Mix fully and incubate at 37°C for 3 min.

④ Add 200 μL of reaction working solution to each well.

⑤ Measure the OD value of each well at 340 nm with microplate reader, recorded as A_1. 3 min later, measure the OD value of each well at 340 nm with microplate reader, recorded as A_2, $\triangle A = A_1 - A_2$.

Note: The control wells measure the total enzyme activity, and the sample wells measure the non-specific enzyme activity. After adding the reaction working solution, record the OD value once every minute for 3 min, observe the change of OD value within 3 min to ensure whether is a constant rate of decline.

Calculation

For tissue sample:

Definition: The amount of mitochondrial complex I in 1 g tissue mitochondria protein per 1 minute that catalyze the decomposition of the 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{mitochondrial complex I activity (U/gprot)} = \frac{(\Delta A_{Control} - \Delta A_{Sample})}{6600 \times 0.7} \times V_1 \div T \div V_2 \div C_{pr} \times f \times 10^6$$

[Note]

$\Delta A_{Control}$: The change OD value of control (A_1 - A_2).

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

6600: The molar extinction coefficient of NADH, L/(mol•cm)

0.7: Optical path, cm

V_1: The volume of the reaction system, 0.24 mL.

V_2: The volume of the sample, 0.02 mL.

T: The time of reaction, 3 min.

f: Dilution factor of sample before test.

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

10^6: 1 mol = 10^6 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat lung 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)	32.50	105.00	197.00
%CV	1.0	0.7	0.7

Inter-assay Precision

Three rat lung 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)	32.50	105.00	197.00
%CV	8.2	8.6	8.4

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	56.8	135	188
Observed Conc. (U/L)	58.5	132.3	197.4
Recovery rate (%)	103	98	105

Sensitivity

The analytical sensitivity of the assay is 4.33 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:

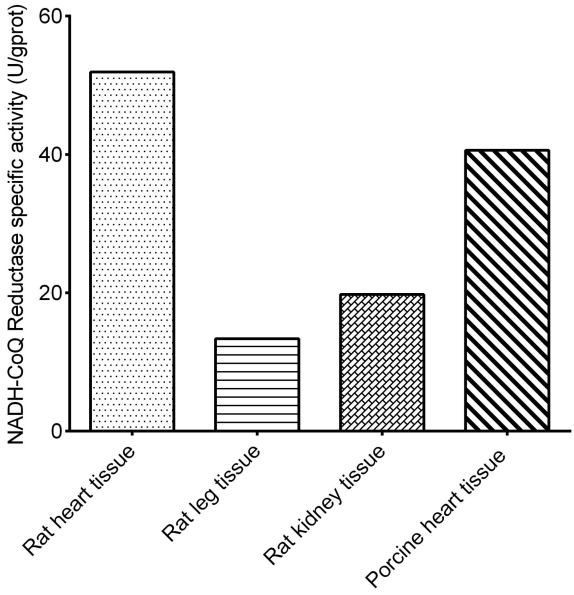
For 20% rat heart tissue homogenate, dilute for 2 times, take 20 μL and carry the assay according to the operation steps. The results are as follows:

the A_1 of the control is 0.597, the A_1 of the sample is 0.722. After 3 minutes, the A_2 of the control is 0.433, the A_2 of the sample is 0.711, $\Delta A_{Control} = A_1 - A_2 = 0.597 - 0.433 = 0.164$, $\Delta A_{Sample} = A_1 - A_2 = 0.722 - 0.711 = 0.011$, the concentration of mitochondria protein in sample is 3.75gprot/L, and the calculation result is:

mitochondrial complex I activity (U/gprot)

$$= \frac{(0.164 - 0.011)}{6600 \times 0.7} \times 0.24 \div 3 \div 0.02 \div 3.75 \times 2 \times 10^6 = 70.65 \text{ U/gprot}$$

Detect 20% rat heart tissue homogenate (the concentration of mitochondria protein is 3.97 gprot/L, dilute for 2 times), 20% rat leg tissue homogenate (the concentration of mitochondria protein is 2.04 gprot/L, dilute for 2 times), 20% rat kidney tissue homogenate (the concentration of mitochondria protein is 7.88 gprot/L, dilute for 4 times) and 20% Porcine heart tissue homogenate (the concentration of protein is 1.54 gprot/L) according to the protocol, the result is as follows:


NADH-CoQ Reductase specific activity (U/gprot)
60
40
20
0
Rat heart tissue
Rat leg tissue
Rat kidney tissue
Porcine heart tissue

Appendix III Publications

1. Li Y, Liu Z, Wang P, et al. Bioengineered Extracellular Vesicles Delivering siMDM2 Sensitize Oxaliplatin Therapy Efficacy in Colorectal Cancer[J]. Advanced Healthcare Materials, 2403531.
2. Tian Y , Hong X , Xie Y ,et al.17 β -Estradiol (E 2) Upregulates the ER α /SIRT1/PGC-1 α 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.
3. 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.
4. 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.
5. Chen L , Chen S , Bai Y ,et al.Electroacupuncture improves cognitive impairment after ischemic stroke based on regulation of mitochondrial dynamics through SIRT1/ PGC-1 α 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.