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

Catalog No：E-BC-K834-M

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

Measuring instrument：Microplate reader(340 nm)

Detection range：2.85-119.04 U/L

Elabscience® Cell 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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 6

The key points of the assay 7

Operating steps 7

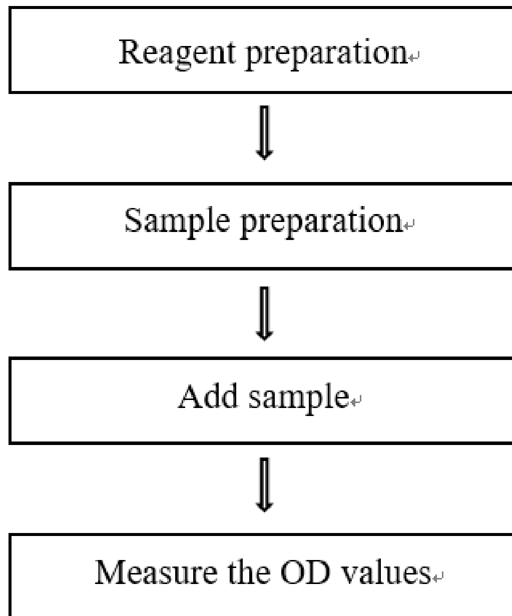
Calculation 8

Appendix I Performance Characteristics 9

Appendix Π Example Analysis 10

Statement 11

Assay summary



Intended use

This kit can measure mitochondrial complex I (NADH-CoQ Reductase) activity in cell samples.

Detection principle

Mitochondrial complex I catalyzes the reaction of NADH with ubiquinone substrate to generate NAD^+ and reduced ubiquinone. The activity of mitochondrial complex I can be quantified by measure the change OD value at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	25 mL ×1 vial	50 mL ×1 vial	-20 ℃, 12 months
Reagent 2	Protease Inhibitor	0.8 mL ×1 vial	0.8 mL ×2 vials	-20 ℃, 12 months, shading light
Reagent 3	Buffer Solution	15 mL ×1 vial	15 mL ×2 vials	-20 ℃, 12 months, shading light
Reagent 4	Substrate A	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months, shading light
Reagent 5	Substrate B	Powder ×1 vial	Powder ×2 vials	-20 ℃, 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 ℃ 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. Store at 2-8 ℃ for 7 days protected from light.

③ The preparation of substrate A working solution：

Before testing, please prepare sufficient substrate A working solution according to the test wells. For example, prepare 505 μL of substrate A working solution (mix well 5 μL of substrate A solution and 500 μL of buffer solution). Store at 2-8 ℃ 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 ℃ for 48 h protected from light, aliquoted storage at -20 ℃ 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, prepare 300 μL of reaction working solution (mix well 5 μL of substrate B working solution and 295 μL of substrate A working solution). The reaction working solution should be prepared on spot and keep it on ice for use within 1 h.

Sample preparation

① Sample preparation

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution and 4 μL protease inhibitor with a ultrasonic cell disruptor (4 ℃, 200W, 5 s/time, interval for 10 s, repeat 15 times).

④ Centrifuge at 10000×g for 3 min at 4 ℃ to remove to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ 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
Jurkat cell	1
CHO cell	1
HL-60cell	1
Hela cell	1
293T cell	1
Molt-4 cell	1

Note: The diluent is extraction solution. 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 or ultrasound.

② During sample measurement, if the OD value decreases by more than 0.3 within 3 min, the sample should be diluted.

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 Sample well: Add 20 μL of sample to the corresponding wells.
Blank well: Add 20 μL of double distilled water to the corresponding wells.

② Add 200 μL of reaction working solution to each well.

③ 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, $\Delta A = A_1 - A_2$.

Calculation

The sample:

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

mitochondrial complex I activity (U/gprot) =

$$\frac{\Delta A_{Sample} - \Delta A_{Blank}}{6600 \times 0.7} \times V_1 \div T \div V_2 \div C_{pr} \times f \times 10^6$$

[Note]

ΔA_{Blank}: The change OD value of blank (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, 0.22 mL.

V_2: The volume of the sample, 0.02 mL.

T: The time of reaction, 5 min.

f: Dilution factor of sample before test.

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

10^6: 1 mol = 10^6 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three Molt-4 cell 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)	30.00	60.00	90.00
%CV	3.0	3.8	4.0

Inter-assay Precision

Three Molt-4 cell 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)	30.00	60.00	90.00
%CV	5.0	6.5	8.0

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 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	20	50	100
Observed Conc. (U/L)	19.0	48.5	105.0
Recovery rate (%)	95	97	105

Sensitivity

The analytical sensitivity of the assay is 2.85 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

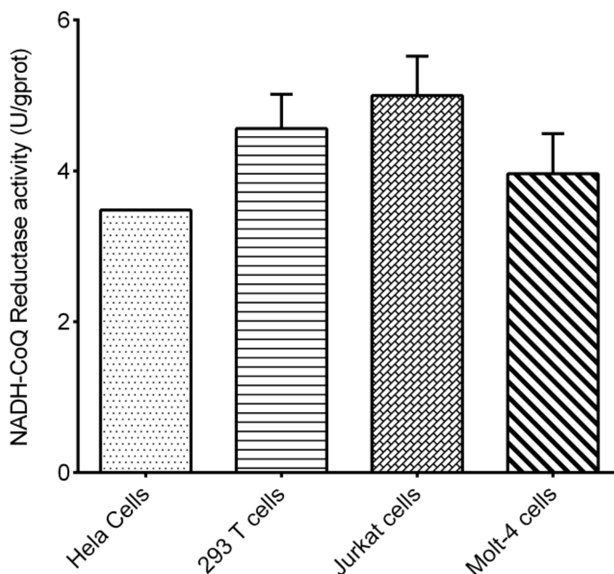
Example analysis：

Take 20 μL Hela cell for detection, and carry the assay according to the operation steps. The results are as follows:

the A_1 of the blank is 0.634, the A_1 of the sample is 0.672. After 5 minutes, the A_2 of the blank is 0.630, the A_2 of the sample is 0.66, $\Delta A_{Blank} = A_1 - A_2 = 0.634 - 0.630 = 0.004$, $\Delta A_{Sample} = A_1 - A_2 = 0.672 - 0.66 = 0.012$, the concentration of mitochondria protein in sample is 3.75 gprot/L and the calculation result is:

$$\text{mitochondrial complex I activity (U/gprot)} = \frac{0.012 - 0.004}{6600 \times 0.7} \times 0.22 \div 5 \div 0.02 \div 1.641 \times 10^6 = 2.32 \text{ U/gprot}$$

Detect Hela (1×10^6, the concentration of protein is 1.641 gprot/L), 293T cell (1×10^6, the concentration of protein is 1.044 gprot/L), Jurkat cell (1×10^6, the concentration of protein is 1.021 gprot/L) and Molt-4 cell (1×10^6, the concentration of protein is 0.627 grot/L, dilute for 10 times) according to the protocol, the result is as follows：



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.