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

Catalog No：E-BC-K836-M

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

Measuring instrument：Microplate reader（540-560 nm）

Detection range：0.73-29.3 U/L

Elabscience® Cell 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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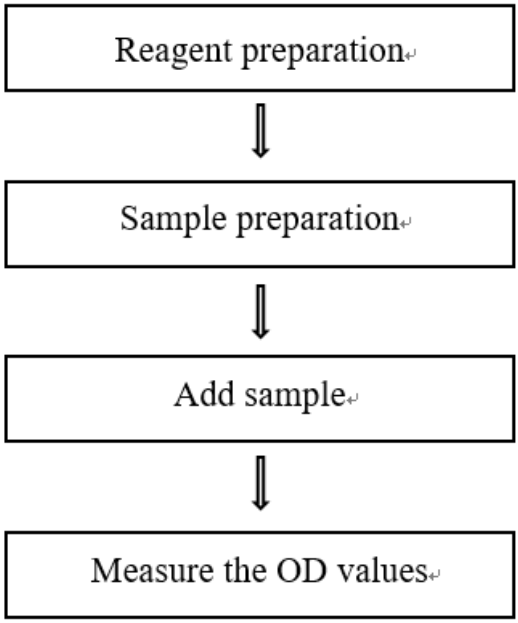
Assay summary



Intended use

This kit can measure mitochondrial complex III (Coenzyme Q-Cytochrome C Reductase) activity in cell 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 Q_{10} formed by mitochondrial complexes I and II to oxidative coenzyme Q_{10}. 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	25 mL ×1 vial	50 mL ×1 vial	-20℃, 12 months
Reagent 2	Inhibitor	0.8 mL ×1 vial	0.8 mL ×2 vials	-20℃, 12 months, shading light
Reagent 3	Substrate A	3 mL ×1 vial	6 mL ×1 vial	-20℃, 12 months, shading light
Reagent 4	Diluent	7 mL×1 vial	14 mL×1 vial	-20℃, 12 months
Reagent 5	Substrate B	0.8 mL×1 vial	1.6 mL×1 vial	-20℃, 12 months, shading light
Reagent 6	Stabilizer	Powder×3 vials	Powder×6 vials	-20℃, 12 months, shading light
Reagent 7	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:

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

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② Place the substrate B at 37℃ for 10 min before use, and mix the solution until its clarified for use. The substrate B can be divided into smaller packages at -20℃ for 1 month.

③ The preparation of stabilizer working solution：

Dissolve one vial of stabilizer with 2 mL of diluent, mix well to dissolve. Store on ice protected from light and used up within 6 h.

④ The preparation of substrate B working solution：

Before testing, please prepare sufficient substrate B working solution according to the test wells. For example, prepare 90 μL of substrate B working solution (mix well 30 μL of stabilizer working solution and 60 μL of substrate B). Stand the prepared solution at room temperature with shading light for 3 min, then use immediately. And the substrate B working solution should be used within 30 min.

⑤ The preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 280 μL of reaction working solution (mix well 20 μL of substrate B working solution, 180 μL of buffer solution and 80 μL of substrate A).

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 of extraction solution and 4 μL of inhibitor, sonicated for 1 min (4℃, 200W, 5 s/time, interval for 10 s, repeat 15 times), centrifuged at 10000×g at 4℃ for 3 min. Then take the supernatant for detection.

④ 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
1×10^6 Jurkat cells	1
1×10^6 Hela cells	1
4×10^6 K562 cells	1
3×10^6 4T1 cells	1
6×10^6 CHO cells	1
1×10^6 293T cells	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 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 reaction working solution, 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.

④ The reaction working solution must be slightly mixed before use, and cannot be shaken violently, otherwise it will precipitate.

Operating steps

① Blank well: Add 40 μL of extraction solution to the corresponding wells.
Sample well: Add 40 μL of sample to the corresponding wells

② Add 240 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s. Measure the OD value of each well at 10 s and 5 min respectively at 550 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_2 - A_1$.

Calculation

Cell sample:

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

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

[Note]

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

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

V_1: The volume of the reaction system, 0.28 mL.

V_2: The volume of the sample, 0.04 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, 5 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 4T1 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)	2.50	15.00	23.00
%CV	5.0	4.1	3.5

Intra-assay Precision

Three 4T1 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)	2.50	15.00	23.00
%CV	4.5	10.0	6.5

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)	8	14	26
Observed Conc. (U/L)	7.9	14.6	26.8
Recovery rate (%)	99	104	103

Sensitivity

The analytical sensitivity of the assay is 0.73 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

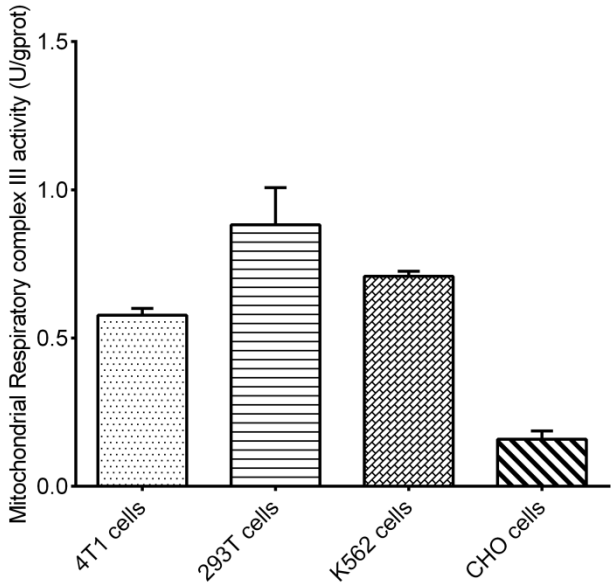
Example analysis:

For 4T1 cell mitochondria supernatant, take 20 μL of supernatant and carry the assay according to the operation table. The results are as follows:

The A_1 of the blank well is 0.563, the A_2 of the blank well is 0.564, ΔA_{blank} = 0.001, The A_1 of sample well is 0.625, the A_2 of sample well is 0.638, ΔA_{sample}= 0.013. The concentration of mitochondria protein in sample is 3.18 gprot/L, and the calculation result is:

$$\text{mitochondrial complex III activity (U/gprot)} = \frac{(0.013 - 0.001) \times 0.28}{0.04 \times 0.0191 \times 0.5 \times 5} \div 3.18 = 0.553 \text{ U/gprot}$$

Detect 3×10^6 4T1cells (the concentration of mitochondria protein is 3.18 gprot/L), 1×10^6 293T cells (the concentration of mitochondria protein is 2.91 gprot/L), 4×10^6 K562 cells (the concentration of mitochondria protein is 4.45 gprot/L) and 6×10^6 CHO cells (the concentration of protein is 7.83gprot/L) 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.