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

Catalog No：E-BC-K837-M

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

Measuring instrument：Microplate reader（540-560 nm）

Detection range：0.79-47.0 U/L

Elabscience® Cell 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.

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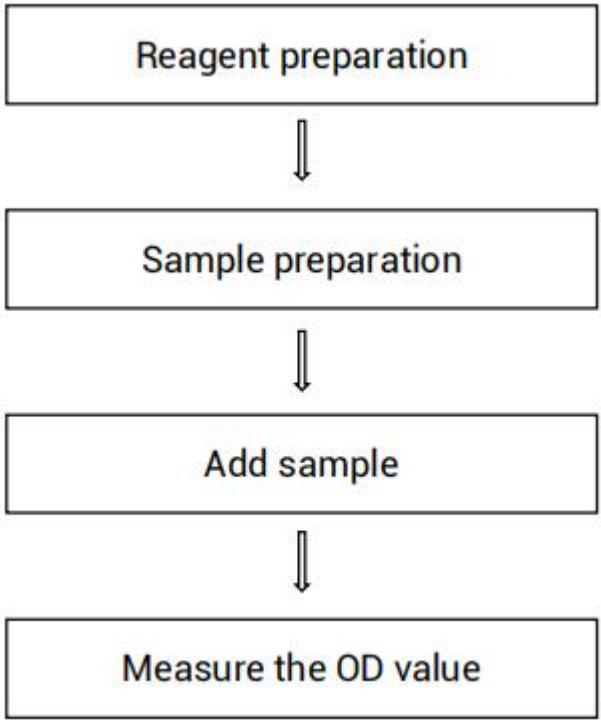
Assay summary



Intended use

This kit can measure mitochondrial complex IV (cytochrome C oxidase) activity in cell 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	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	Powder × 2 vials	Powder × 4 vials	-20℃, 12 months, shading light
Reagent 4	Stabilizer	Powder × 2 vials	Powder × 4 vials	-20℃, 12 months, shading light
Reagent 5	Buffer Solution	13 mL ×1 vial	26 mL ×1 vial	-20℃, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout 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:

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

Reagent preparation

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

② The preparation of substrate working solution A：
Dissolve one vial of substrate with 4 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 1 month protected from light, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of stabilizer working solution：
Dissolve one vial of stabilizer with 200 μL of buffer solution, mix well to dissolve. Storage at -20°C for 1 month protected from light, and avoid repeated freeze/thaw cycles is advised.

④ The preparation of reaction working solution：
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 2003 μL of substrate working solution (mix well 2000 μL of substrate working solution A and 3 μL of stabilizer working solution). Stand the prepared solution at room temperature with shading light for 15 min, then use immediately. And the substrate working solution should be used within 4 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 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°C for 10 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
4×10^6 CHO cells	1
1×10^6 K562 cells	1
1×10^6 HL-60 cells	1
1×10^6 Hela 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 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. If it exceeds this range, it is recommended to extend the standing time of the reaction working solution.

Operating steps

① Blank well: Add 40 μL of extraction solution to blank well.
Sample well: Add 40 μL of sample to sample well.

② Add 140 μL of reaction working solution to each well.

③ Measure the OD value of each well at 550 nm with microplate reader at 10 s and 3 min 10 s (Incubate at 37°C) respectively recorded as A_1 and A_2, $\Delta A = A_1 - A_2$.

Calculation

For cell sample:

Definition: The amount of mitochondrial complex IV in 1 g cell mitochondrial protein per 1 minute that oxidize 1 μmol of cytochrome C at 37°C 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, 0.18 mL.

V_2: The volume of the sample, 0.04 mL.

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

d: Optical path, 0.5 cm

T: The time of reaction, 3 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 hela 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)	5.50	18.00	27.00
%CV	5.5	3.5	3.0

Intra-assay Precision

Three hela 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)	5.50	18.00	27.00
%CV	9.5	4.0	5.1

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	12	20	25
Observed Conc. (U/L)	12.5	20.2	25.3
Recovery rate (%)	104	101	101

Sensitivity

The analytical sensitivity of the assay is 0.79 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

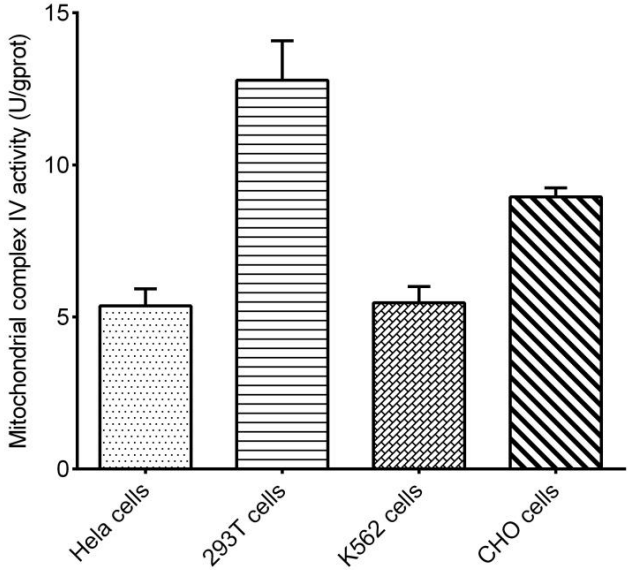
Example analysis：

For Hela cell mitochondria supernatant, take 40 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 0.623, the A_2 of the blank well is 0.625. The A_1 of sample well is 0.680, the A_2 of sample well is 0.624. The concentration of mitochondria protein in sample is 1.641 gprot/L, and the calculation result is:

$$\text{mitochondrial complex IV (U/gprot)} = \frac{((0.680 - 0.624) - (0.623-0.625)) \times 0.18}{0.04 \times 0.0191 \times 0.5 \times 3} \div 1.641 = 5.55 \text{ U/gprot}$$

Detect 1×10^6 4T1cells (the concentration of mitochondria protein is 1.64 gprot/L), 1×10^6 293T cells (the concentration of mitochondria protein is 1.04 gprot/L), 1×10^6 K562 cells (the concentration of mitochondria protein is 1.30 gprot/L) and 4×10^6 CHO cells (the concentration of protein is 5.51gprot/L) according to the protocol, the result is as follows：


Mitochondrial complex IV activity (U/gprot)
15
10
5
0
Hela cells
293T cells
K562 cells
CHO cells

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.