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

Catalog No：E-BC-K838-M

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

Measuring instrument：Microplate reader（330-350 nm）

Detection range：3.40-272.07 U/L

Elabscience® Cell Mitochondrial Complex V (F_0F_1-ATPase/ATP Synthase) 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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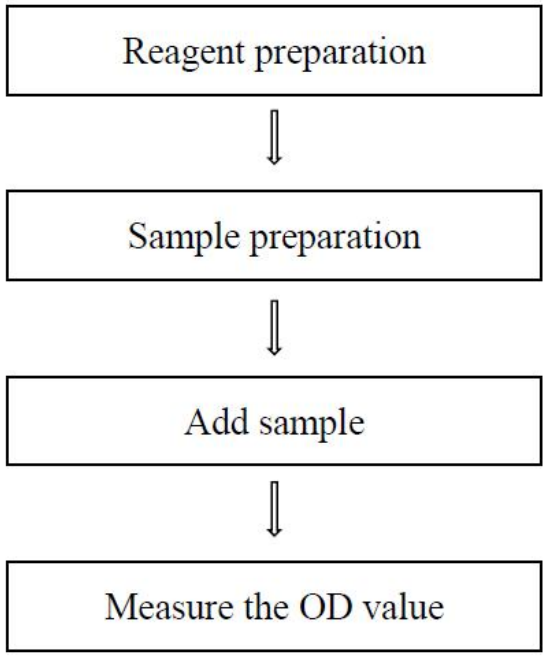
Assay summary



Intended use

This kit can measure the activity of mitochondrial complex V (F_0F_1-ATPase/ATP Synthase) in cell sample.

Detection principle

Mitochondrial complex V is also known as F_0F_1-ATP synthase. ATP is hydrolyzed by F_0F_1-ATP synthase to produce ADP, and ADP converts NADH into oxidized coenzyme I (NAD) after enzyme conversion reaction. The activity of mitochondrial complex V 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	30 mL × 1 vial	-20℃, 12 months
Reagent 4	Substrate A	Liquid × 1 vial	Liquid × 2 vials	-20℃, 12 months, shading light
Reagent 5	Substrate B	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 6	Substrate C	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 7	Substrate D	Powder × 2 vials	Powder × 4 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:

Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate B working solution:

Dissolve one vial of substrate B with 300 μL of double distilled water, mix well. Aliquot and store at -20°C for 7 days protected from light.

③ The preparation of substrate C working solution:

Dissolve one vial of substrate C with 300 μL of double distilled water, mix well. Aliquot and store at -20°C for 7 days protected from light.

④ The preparation of reaction working solution:

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 525 μL of reaction working solution (mix well 500 μL of buffer solution, 5 μL of Substrate A, 10 μL of Substrate B working solution, 10 μL of Substrate C working solution). The prepared solution can be stored at 2-8°C for 8 h protected from light.

⑤ The preparation of enzyme working solution:

Dissolve one vial of substrate D with 0.6 mL of double distilled water, mix well. Store at 2-8°C for 3 days protected from light.

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 protease inhibitor, mix fully and sonicated for 1 min (4℃, 200W, 5 s/time, interval for 10 s, repeat 15 times).

④ Centrifuge at 10000×g for 3 min at 4°C. Then take the supernatant 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
1×10^6 HL-60 cells	1
1×10^6 Hela cells	1
4×10^6 CHO cells	1
1×10^6 Jurkat 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 During reagent preparation, it is necessary to ensure that the powder is completely dissolved in the reaction working solution after preparation.

② The detection is started at about 10 s after adding reaction working solution.

③ For sample detection, if the A_1 of the sample well is lower than 0.7, or the change OD value (ΔA) of the sample well for 4 minutes exceeds 0.3, the sample should be diluted.

Operating steps

① Blank well: Add 20 μL of extraction solution to the control wells.
Sample well: Add 20 μL of sample to the sample wells.

② Add 20 μL of enzyme working solution to each well.

③ Add 180 μL of reaction working solution to each well.

④ Measure the OD value of each well at 340 nm with microplate reader, recorded as A_1. After 4 min, measure the OD value of each well at 340 nm with microplate reader, recorded as A_2. $\triangle A = A_1 - A_2$. (It is recommended to follow the notes below for measurement)

Note: After adding the reaction working solution, record the OD value once every minute for 4 min, observe the change of OD value within 4 min to ensure whether is a constant rate of decline. Otherwise, the sample needs to be diluted. When calculating, take initial OD value A_1, A_2 OD value after 4 min.

Calculation

For cell sample:

Definition: The amount of mitochondrial complex V in 1 g mitochondrial protein per 1 minute that catalyze decomposition of 1 μmol NADH at 37℃ is defined as 1 unit.

$$\text{mitochondrial complex V activity (U/gprot)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{6220 \times 0.65} \times 0.22 \div t \div 0.02 \div C_{pr} \times f \times 10\text{^}6$$

[Note]

ΔA_{sample}: The change OD value of sample well, A_1-A_2.

ΔA_{blank}: The change OD value of blank well, A_1-A_2.

6220: Molar absorption coefficient, L/(mol*cm).

0.65: Optical path, cm.

0.22: The volume of the reaction system, mL.

0.02: The volume of the sample, mL.

T: The time of reaction, 4 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 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)	20.00	40.00	70.00
%CV	3.0	3.4	4.0

Inter-assay Precision

Three cell serum 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)	20.00	40.00	70.00
%CV	4.2	3.0	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 103%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	20	40	70
Observed Conc. (U/L)	20.2	42	71.4
recovery rate (%)	101	105	102

Sensitivity

The analytical sensitivity of the assay is 3.40 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

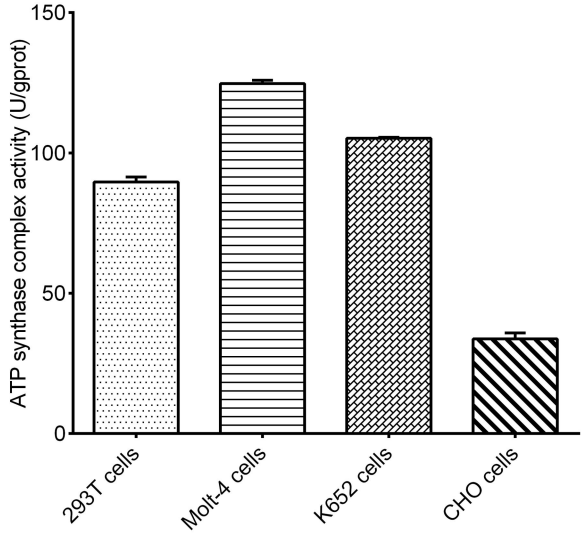
Example analysis：

For 293T cell supernatant, carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 0.752, the A_2 of the sample well is 0.621, $\triangle A_{sample}$ = 0.752 - 0.621 = 0.131. The A_1 of blank well is 0.634, the A_2 of blank well is 0.630, $\triangle A_{blank}$ = 0.634 - 0.630 = 0.004, the concentration of protein in sample is 1.044 gprot/L, and the calculation result is:

$$\text{mitochondrial complex V activity (U/gprot)} = \frac{0.131 - 0.004}{6220 \times 0.65} \times 0.22 \div 4 \div 0.02 \div 1.044 \times 10^6 = 82.743 \text{ U/gprot}$$

Detect 293T cells (the concentration of protein is 1.004 gprot/L), Molt-4 cells (the concentration of protein is 0.627 gprot/L), K652 cells (the concentration of protein is 1.300 gprot/L) and CHO cells (the concentration of protein is 5.510 gprot/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.