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

Catalog No：E-BC-K831-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（630-640 nm）

Detection range：0.06 - 2.75 U/L

Elabscience®ATPase 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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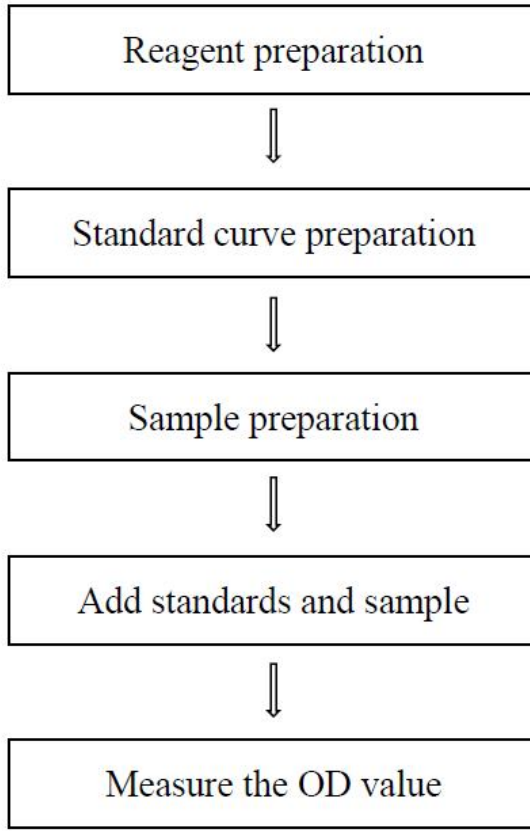
Assay summary



Intended use

This kit can measure ATPase activity in animal tissue and cell samples.

Detection principle

ATPase, also known as adenosine triphosphate, is an important high-energy compound in organisms, which can maintain the balance of cell membrane potential and ions.

ATPase catalyzes the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphorus. The activity of ATPase was determined by inorganic phosphorus production per unit time.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Acid Reagent	25 mL × 1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent A	12 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 5	Chromogenic Agent B	4 mL × 1 vial	2-8°C, 12 months
Reagent 6	10 mmol/L Standard	0.5 mL × 1 vial	2-8°C, 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:

Vortex mixer, Incubator, Centrifuge, Microplate reader (630-640 nm, optimum wavelength: 640 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：

Dissolve one vial of substrate with 10 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 7 days protected from light.

③ Preparation of chromogenic working solution：

For each well, prepare 100 μL of chromogenic working solution (mix well 75 μL of chromogenic agent A and 25 μL of chromogenic agent B), and stand at 37℃ for 1 h. The prepared solution should be prepared on spot and used up within 10 hours.

④ Preparation of 0.1 mmol/L standard：

Dliute 10 μL of 10 mmol/L standard with 990 μL of double distilled water, mix well. The prepared solution should be prepared on spot and stored at 2-8°C.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.00, 0.01, 0.02, 0.03, 0.05, 0.06, 0.08, 0.10 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.01**	**0.02**	**0.03**	**0.05**	**0.06**	**0.08**	**0.1**
0.1 mmol/L standard (μL)	0	20	40	60	100	120	160	200
Double distilled water (μL)	200	180	160	140	100	80	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

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

③ Homogenize 2×10^6 cells in 400 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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
10% Rat liver tissue homogenate	5-10
10% Rat kidney tissue homogenate	5-10
10% Rat heart tissue homogenate	5-10
10% Rat spleen tissue homogenate	5-10
10% Rat lung tissue homogenate	5-10
10% Mouse liver tissue homogenate	5-10
10% Mouse spleen tissue homogenate	5-10
10% Mouse lung tissue homogenate	5-10
10% Mouse kidney tissue homogenate	5-10
293T cell	1

Note: The diluent is normal saline (0.9%NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

Enzymatic reaction

① Control tube: Take 160 μL of double distilled water to the 1.5 mL EP tube.
Sample tube: Take 160 μL of double distilled water to the 1.5 mL EP tube.

② Add 100 μL of sample to the sample tube.

③ Add 260 μL of buffer solution to each tube.

④ Add 80 μL of substrate working solution to each tube.

⑤ Mix fully and incubate at 37℃ for 30 min.

⑥ Add 100 μL of acid reagent to each tube and mix fully.

⑦ Add 100 μL of sample to the control tube. Mix fully and centrifuge at 3500×g for 10 min, then take the supernatant for detection.

Chromogenic reaction

① Standard well: Add 30 μL of standard solution with different concentrations to the corresponding wells.
Control well: Add 30 μL of supernatant from control tube to the corresponding wells.
Sample well: Add 30 μL of supernatant from sample tube to the corresponding wells.

② Add 100 μL of chromogenic working solution to each well.

③ Mix fully with microplate reader for 5 s and incubate at room temperature for 2 min.

④ Add 100 μL of acid reagent to each well.

⑤ Mix fully with microplate reader for 5 s and incubate at room temperature for 10 min with shading light. Measure the OD value of each well at 640 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue and cell sample:

Definition: The amount of ATPase in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol of product at 37°C is defined as 1 unit.

$$\underset{\text{(U/gprot)}}{\text{ATPase activity}} = \frac{(\Delta A - b)}{a} \div C_{pr} \div T \times f \times 1000^{*}$$

[Note]

ΔA: $OD_{sample} - OD_{control}$.

T: The time of incubation reaction, 30 min.

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

f: Dilution factor of sample before test.

1000*: 1 mmol/L = 1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	0.85	1.36	2.25
%CV	4.4	3.8	3.8

Inter-assay Precision

Three human 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)	0.85	1.36	2.25
%CV	4.6	5.2	5.2

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 95%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.015	0.04	0.075
Observed Conc. (mmol/L)	0.0	0.0	0.1
Recovery rate(%)	96	98	91

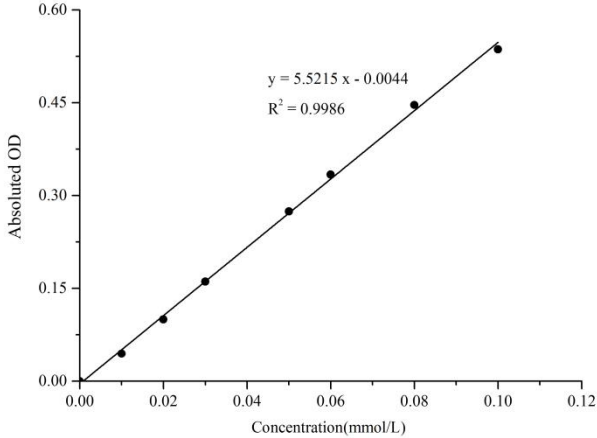
Sensitivity

The analytical sensitivity of the assay is 0.06 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.01**	**0.02**	**0.03**	**0.05**	**0.06**	**0.08**	**0.10**
Average OD	0.048	0.092	0.148	0.209	0.322	0.382	0.494	0.580
Absoluted OD	0.000	0.045	0.100	0.161	0.275	0.334	0.447	0.540



Appendix II Example Analysis

Example analysis：

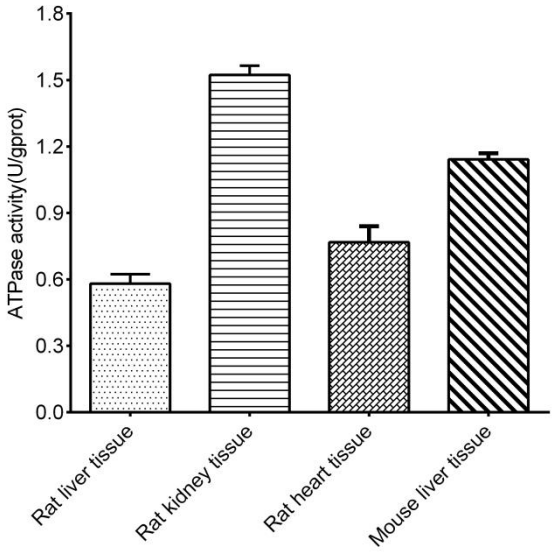
For rat liver tissue, take 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 5.5215 x – 0.0044, the OD value of the control is 0.639, the OD value of the sample is 0.784, the concentration of protein in sample is 7.75 gprot/L, and the calculation result is:

$$\text{ATPase activity (U/gprot)} = (0.784 - 0.639 + 0.0044) \div 5.5215 \div 7.75 \div 30 \times 1000 \times 5$$

$$= 0.62 \text{ U/gprot}$$

Detect 10% rat liver tissue homogenate (the concentration of protein is 7.75 gprot/L, dilute for 5 times), 10% rat kidney tissue homogenate (the concentration of protein is 6.85 gprot/L, dilute for 5 times), 10% rat heart tissue homogenate (the concentration of protein is 4.80 gprot/L, dilute for 5 times) and 10% mouse liver tissue homogenate (the concentration of protein is 9.05 gprot/L, dilute for 5 times), according to the protocol, the result is as follows：



Appendix III Publications

1. Ouyang B , Zhong Q , Ouyang P ,et al.Graphene quantum dots enhance the biological nitrogen fixation by up-regulation of cellular metabolism and electron transport[J].Chemical Engineering Journal, 2024, 487.DOI:10.1016/j.cej.2024.150694.
2. Wang C , Zhao H , Liu Y ,et al.Neurotoxicity of manganese via ferroptosis induced by redox imbalance and iron overload[J].Ecotoxicology and Environmental Safety, 2024, 278(000):19.DOI:10.1016/j.ecoenv.2024.116404.
3. Li J , Zhang D , Zhang Y ,et al.Mitochondria‐specific antioxidant MitoTEMPO alleviates senescence of bone marrow mesenchymal stem cells in ovariectomized rats[J].Journal of Cellular Physiology, 2024, 239(8).DOI:10.1002/jcp.31323.
4. Chen X , Yang T , Zhou Y ,et al.Astragaloside IV combined with ligustrazine ameliorates abnormal mitochondrial dynamics via Drp1 SUMO/deSUMOylation in cerebral ischemia‐reperfusion injury[J].CNS Neuroscience & Therapeutics, 2024, 30(4).DOI:10.1111/cns.14725.
5. Gao X , Zhang R , Wang Z ,et al.Preliminary study on the protective effect of remazolam against sepsis-induced acute respiratory distress syndrome (ARDS)[J].PeerJ, 2024.

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.