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

Catalog No：E-BC-K122-S

Specification：100Assays(50 samples)/200Assays(100 samples)

Measuring instrument：Spectrophotometer（660 nm）

Elabscience® H^+K^+-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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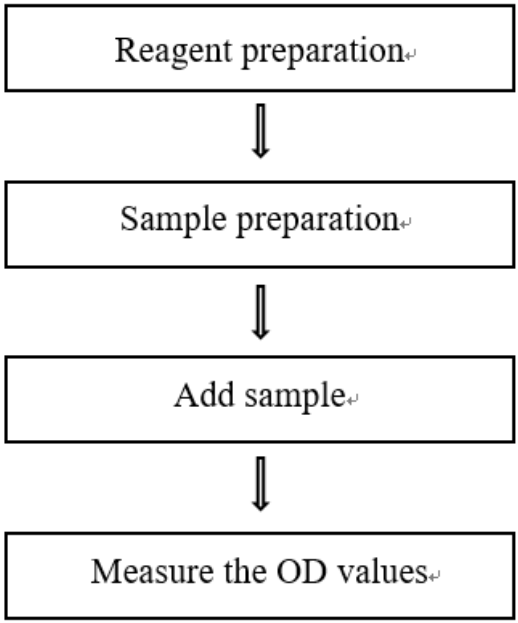
Assay summary



Intended use

The kit is used for the determination of H+K+-ATPase activity in animal tissue and cells samples.

Detection principle

ATPase can decompose ATP to produce ADP and inorganic phosphorus. The activity of ATPase can be expressed by measuring the production amount of inorganic phosphorus in unit time. The inorganic phosphorus reacts with ammonium molybdate in acidic solution to form ammonium molybdate compound, which is reduced with reducing agent to form molybdenum blue, and has absorption peak at 660 nm. Determine the concentration of molybdenum blue to calculate the amount of inorganic phosphorus.

Kit components & storage

Item	Component	Size 1 (100 Assays)	Size 2 (200 Assays)	Storage
Reagent 1	Buffer Solution	20 mL ×1 vial	40 mL ×1 vial	2-8 ℃, 12 months
Reagent 2	Accelerator	8 mL × 1 vial	16 mL × 1 vial	2-8 ℃, 12 months
Reagent 3	Acid Solution	8 mL × 1 vial	16 mL × 1 vial	2-8 ℃, 12 months
Reagent 4	Substrate	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months
Reagent 5	Inhibitor	Powder ×1 vial	Powder ×2 vials	2-8 ℃, 12 months
Reagent 6	Complexing Agent	6 mL ×1 vial	12 mL ×1 vial	2-8 ℃, 12 months
Reagent 7	Stop Solution	10 mL ×1 vial	10 mL ×2 vials	2-8 ℃, 12 months
Reagent 8	Reducing Agent	Powder ×2 vials	Powder ×3 vials	2-8 ℃, 12 months, shading light
Reagent 9	Chromogenic Agent	Powder ×1 vial	Powder ×2 vials	2-8 ℃, 12 months
Reagent 10	2.5 mol/L Sulphuric Acid	60 mL ×1 vial	60 mL ×2 vials	2-8 ℃, 12 months
Reagent 11	Standard Stock Solution	10 mL ×1 vial	10 mL ×1 vial	2-8 ℃, 12 months

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:

Spectrophotometer (660 nm), Micropipettor, Centrifuge, Incubator, Water bath, Vortex mixer

Consumptive material:

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate application solution:
Dissolve one vial of substrate with 5 mL of double distilled water, mix well. Store at -20 ℃ for 1 week.

③ The preparation of inhibitor application solution:
Dissolve one vial of inhibitor with 5 mL of double distilled water, mix well. Store at 2-8 ℃ for 1 week.

④ The preparation of stop application solution:
Dissolve one vial of stop solution with 15 mL of double distilled water, mix well. Store at 2-8 ℃ for 3 months.

⑤ The preparation of reducing application solution:
Dissolve one vial of reducing agent with 30 mL of double distilled water, Mix well. Store at 2-8 ℃ for 1 week protected from light.

⑥ The preparation of chromogenic application solution:
Dissolve one vial of chromogenic with 60 mL of double distilled water, Mix well. Store at 2-8 ℃ for 3 months. If there is a small amount of insoluble powder, take supernatant directly, it will not affect the results.

⑦ The preparation of phosphorus assay reagent:
For each well, prepare 2000 μL of phosphorus assay reagent (mix well 800 μL of double distilled water, 400 μL of 2.5 mol/L sulphuric acid, 400 μL of reducing application solution and 400 μL of chromogenic application solution) Store at 2-8 ℃ for 1 day protected from light.

⑧ The preparation of 0.5 μmol/mL standard:
Dilute 20 μL of standard stock solution with 380 μL of double distilled water, mix well. Store at 2-8 ℃ for 1 day.

⑨ The preparation of working solution A:

For each well, prepare 330 μL of working solution A (mix well 130 μL of buffer solution, 120 μL of acid solution, 40 μL of substrate application solution and 40 μL of inhibitor application solution).

⑩ The preparation of working solution B:

For each well, prepare 330 μL of working solution A (mix well 130 μL of buffer solution, 80 μL of accelerator, 40 μL of substrate application solution, 40 μL of inhibitor application solution and 40 μL of complexing agent).

Sample preparation

① Sample preparation

Sample preparation

Do not treat the samples with phosphorus-containing reagents and detergents such as SDS, Tween20, NP-40, Triton X-100.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

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

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 min 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 1×10^6 cells).

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

③ Homogenize 1×10^6 cells in 300-500 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min 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% Animal tissue homogenate	5
GES-1 cells (2.52mg/mL)	2

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

The key points of the assay

① The tubes used in assay must be disposed strictly without a trace of phosphorus. It is better to use disposable tubes or new tubes to avoid pollution of phosphorus which is the key for success.

② All the containers of reagents should be dedicated, including the pipette of drawing sulfuric acid and distilled water containers.

③ The protein concentration of the sample to be tested should be less than 3 mg/mL.

Operating steps

1. Enzymatic reaction

① Control tube: add 330 μL of working solution A to 1.5 mL EP tube.
Sample tube: add 330 μL of working solution B to 1.5 mL EP tube.

② Add 100 μL of sample to sample tube.

③ Mix fully and incubate at 37 ℃ for 10 min.

④ Add 50 μL of stop application solution to each tube.

⑤ Add 100 μL of sample to control tube.

⑥ Mix fully and centrifuge at 2000×g for 10 min, take 400 μL supernatant of each tube for phosphorus assay.

2. Phosphorus assay

① Standard tube: add 400 μL of 0.5 μmol/mL standard to 5 mL EP tube
Control tube: add 400 μL of supernatant from corresponding control tube to 5 mL EP tube.
Sample tube: add 400 μL of supernatant from corresponding sample tube to 5 mL EP tube.

② Add 2000 μL of phosphorus assay reagent to each tube.

③ Mix fully, incubate at 45 ℃ for 10 min and cool to room temperature.

④ Set the spectrophotometer to zero with distilled water and measure the OD of each tube at 660 nm with 1 cm optical path quartz cuvette.

Calculation

The sample:

Definition: 1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 1 mg of tissue protein per hour is defined as 1 ATPase activity unit.

$$\underset{(\mu mol\ Pi/mgprot/hour)}{H^+K^+\text{-ATPase activity}} = \frac{A_2 - A_1}{A_3} \times C \times 4.8^* \times 6^{**} \div C_{pr} \times f$$

[Note]

A_1: the OD value of control

A_2: the OD value of sample

A_3: the OD value of standard

C: the concentration of standard, 0.5 μmol/mL

4.8*: the dilution factor of the sample in the reaction system

$$4.8 = \frac{\text{The total volume of reaction}}{\text{The volume of sample}} = \frac{130 + 80 + 40 + 40 + 40 + 50 + 100}{100}$$

6**: the reaction time is 10 min, but the time in unit definition is an hour.

C_{pr}: Concentration of protein in sample, mgprot/mL

f: Dilution factor of sample before tested.

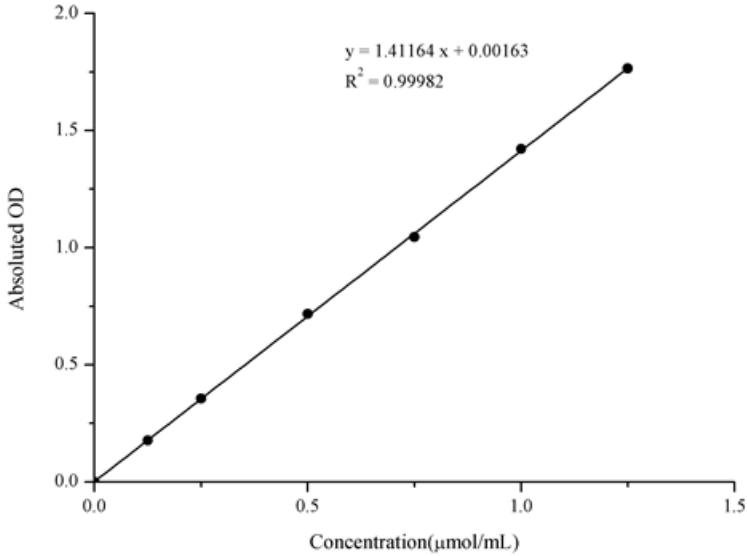
Appendix I Performance Characteristics

Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (μmol/mL)	0	0.125	0.25	0.5	0.75	1	1.25
Average OD	0.002	0.179	0.358	0.719	1.048	1.424	1.767
Absoluted OD	0	0.177	0.356	0.717	1.046	1.422	1.765



Appendix II Example Analysis

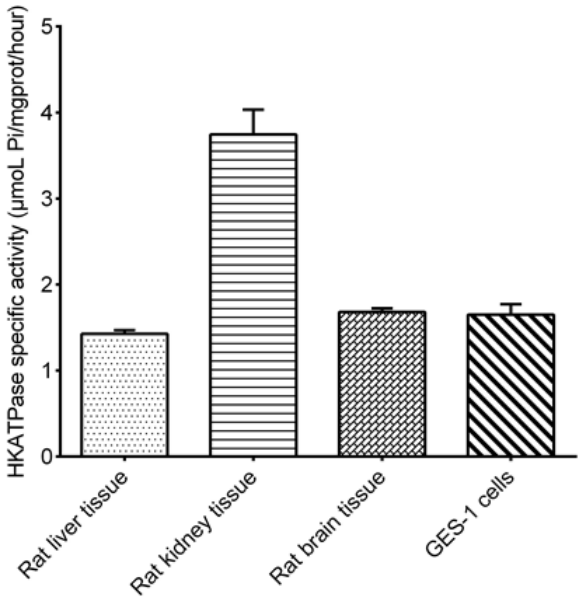
Example analysis:

Dilute 10% rat kidney tissue homogenate with normal saline for 5 times, then take 100 μL of 2% rat kidney tissue homogenate and carry the assay according to the operation steps. The results are as follows:

the average OD value of the control is 0.189, the average OD value of the sample is 0.455, the average OD value of the standard is 0.736, the concentration of protein in sample is 6.95 mgprot/mL, and the calculation result is:

$$\underset{(\mu mol\ Pi/mgprot/hour)}{H^+K^+\text{-ATPase activity}} = \frac{0.455-0.189}{0.736} \times 0.5 \times 4.8 \times 6 \div 6.95 \times 5 = 3.74\ \mu mol\ Pi/mgprot/hour$$

Detect 10% rat liver tissue homogenate (the concentration of protein is 15.87 mgprot/mL, dilute for 5 times), 10% rat kidney tissue homogenate (the concentration of protein is 6.95 mgprot/mL, dilute for 5 times), 10% rat brain tissue homogenate (the concentration of protein is 3.29 mgprot/mL, dilute for 5 times) and GSE-1 cells (the concentration of protein is 2.52 mgprot/mL, dilute for 2 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.