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

Catalog No：E-BC-K212-S

Specification：50 Assays(23 samples)/100 Assays(48 samples)

Measuring instrument：Spectrophotometer（660 nm）

Detection range：0.8-41 U/g wet weight

Elabscience® Ca^{2+}-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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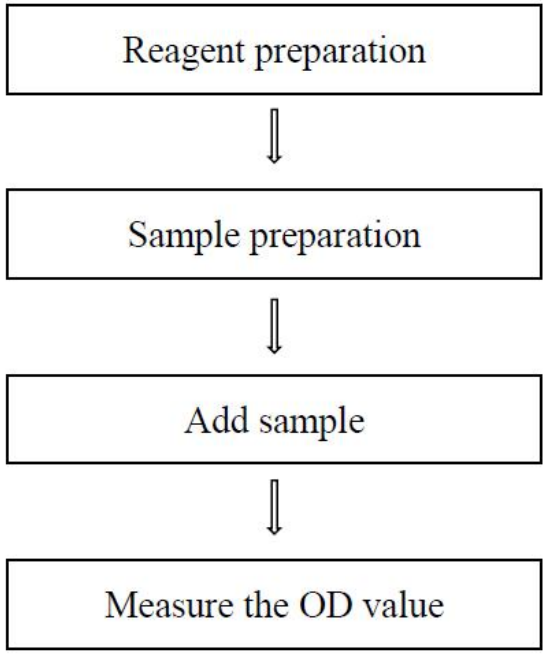
Assay summary



Intended use

This kit can be used for detection of Ca^{2+}-ATPase activity in animal tissue and cell samples.

Detection principle

ATPase can decompose ATP to produce inorganic phosphorus. The activity of ATPase can be expressed by measuring the production amount of inorganic phosphorus in unit time. In the control system, Ca^{2+} -ATPase activity was inhibited, while in the sample system, Ca^{2+} -ATPase activity was not inhibited. The difference of inorganic phosphorus content between the sample and the control was the inorganic phosphorus produced by Ca^{2+} -ATPase during the incubation time. The activity of Ca^{2+} -ATPase was determined by inorganic phosphorus production.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	2-8℃, 12 months
Reagent 2	Activator A	2 mL × 1 vial	2 mL × 2 vials	2-8℃, 12 months
Reagent 3	Activator B	2 mL × 1 vial	2 mL × 2 vials	2-8℃, 12 months
Reagent 4	Substrate	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months
Reagent 5	Protein Precipitator	10 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months shading light
Reagent 7	Acid Agent	25 mL × 1 vial	50 mL × 1 vial	2-8℃, 12 months
Reagent 8	Chromogenic Agent B	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 9	10 mmol/L Standard Solution	2 mL × 1 vial	2 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:

Test tube, Micropipettor, Vortex mixer, Incubator, Centrifuge, Spectrophotometer (660 nm)

Reagents:

Ultrapure water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The 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 a week.

③ The preparation of chromogenic agent A working solution:
Dissolve one vial of chromogenic agent A with 25 mL of double distilled water, mix well to dissolve. Store at 2-8°C for a week protected from light.

④ The preparation of chromogenic agent B working solution:
Dissolve one vial of chromogenic agent B with 25 mL of double distilled water in 90-100°C water bath. Store at 2-8°C for a week.

⑤ 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 chromogenic agent A working solution, 400 μL of acid reagent and 400 μL of chromogenic agent B working solution). Prepared solution should be pale yellow. If it is colorless or blue, it should be invalid or phosphorus pollution. The phosphorus assay reagent should be

prepared on spot and protected from light.

⑥ The preparation of 0.5 μmol/mL standard：

For each well, prepare 200 μL of 0.5 μmol/mL standard (mix well 10 μL of 10 mmol/L standard and 190 μL of double distilled water). The 0.5 μmol/mL standard should be prepared on spot. Store at 2-8°C for a week.

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).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 4×10^6 cells).

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

③ Homogenize 4×10^6 cells in 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% Rat liver tissue homogenate	5-8
10% Rat heart tissue homogenate	5-8
10% Rat kidney tissue homogenate	5-8
10% Mouse liver tissue homogenate	1
10% Rat lung tissue homogenate	5-8
10% Rat brain tissue homogenate	2-3

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 With the preparation of phosphorus assay reagent, glass container can be selected for preparation. After the glass container is repeatedly scrubbed before use, it is repeatedly rinsed 10 times with double steamed water. Prepared solution should be pale yellow. If it is green or blue, it should be invalid or phosphorus pollution and it needs to be re-prepared.

② During the operation, take supernatant for determination carefully, and do not take precipitate.

Operating steps

Enzymatic reaction

① Control tube: add 170 μL of buffer solution to the 1.5 mL EP tube.
Sample tube: add 170 μL of buffer solution to the 1.5 mL EP tube.

② Add 40 μL of activator A to control tube, and add 40 μL of activator B to sample tube.

③ Add 40 μL of substrate working solution to each tube.

④ Add 200 μL of sample to the sample tube, and mix well with vortex mixer.

⑤ Incubate at 37℃ for 10 min.

⑥ Add 50 μL of protein precipitator to control tube, mix fully and add 200 μL of sample. Add 50 μL of protein precipitator to the enzyme tube.

⑦ Mix fully and centrifuge at 2000×g for 10 min, then take the supernatant for detection.

Color reaction

① Standard tube: add 200 μL of 0.5 μmol/mL standard to 5 mL EP tube
Blank tube: add 200 μL of double distilled water to 5 mL EP tube
Control tube: add 200 μL of supernatant from corresponding control tube to 5 mL EP tube.
Sample tube: add 200 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 well, incubate at 37℃ for 30 min. 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:

1. Tissue sample (tissue protein):

Definition: 1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 1 mg of tissue protein per hour at 37℃ is defined as 1 ATPase activity unit.

$$\underset{\text{(U/mgprot)}}{Ca^{2+}\text{-ATPase activity}} = \frac{A_2}{A_1} \times C \div t \times \frac{V_1}{V_2} \div C_{pr} \times f$$

2. Tissue sample (wet weight):

Definition: 1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 1 g of wet weight per hour at 37℃ is defined as 1 ATPase activity unit.

$$\underset{\text{(U/g wet weight)}}{Ca^{2+}\text{-ATP activity}} = \frac{A_2}{A_1} \times C \div t \times \frac{V_1}{V_2} \div \frac{m}{V_3} \times f$$

3. For cell (number of cells):

Definition: 1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 10^6 of cell per hour at 37℃ is defined as 1 ATPase activity unit.

$$\underset{\text{(U/}10^6\text{)}}{Ca^{2+}\text{-ATP activity}} = \frac{A_2}{A_1} \times C \div t \times \frac{V_1}{V_2} \div \frac{N}{V_3} \times f$$

[Note]

A_2: $OD_{Sample} - OD_{Control}$.

A_1: $OD_{Standard} - OD_{Blank}$.

C: The concentration of standard, 0.5 μmol/mL.

t: The time of incubation reaction, 10 min=1/6 h.

V_1: The total volume of incubation reaction, 500 μL.

V_2 The volume of sample, 200 μL.

V_3: The volume of normal saline homogenate, mL.

m: The weight of tissue, g.

C_{pr}: The concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

N: The number of cell sample/10^6.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse kidney 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/kg wet weight)	5.20	18.40	25.50
%CV	4.6	4.2	3.5

Inter-assay Precision

Three mouse kidney samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/kg wet weight)	5.20	18.40	25.50
%CV	8.4	9.0	9.0

Sensitivity

The analytical sensitivity of the assay is 0.8 U/g wet weight. 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：

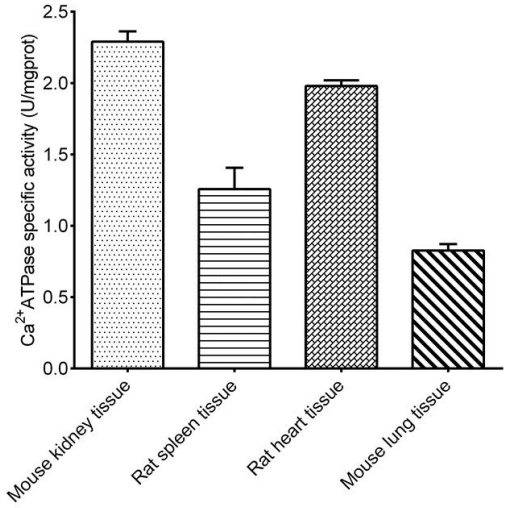
Take 10% mouse kidney tissue homogenate, dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

the OD value of the control tube is 0.152, the OD value of the sample tube is 0.323, the OD value of the standard tube is 0.405, the OD value of the blank tube is 0.001, the concentration of protein in sample is 6.69 mgprot/mL, and the calculation result is:

$$Ca^{2+}\ \text{ATPase activity (U/mgprot)}$$

$$= (0.323\text{-}0.152) \div (0.405 - 0.001) \times 0.5 \times 6 \times 500 \div 200 \times 5 \div 6.69 = 2.37\ \text{U/mgprot}$$

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 6.69 mgprot/mL), 10% rat spleen tissue homogenate (the concentration of protein is 5.39 mgprot/mL dilute for 5 times), 10% rat heart tissue homogenate (the concentration of protein is 6.68 mgprot/mL dilute for 5 times) and 10% mouse lung tissue homogenate (the concentration of protein is 3.90 mgprot/mL dilute for 5 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.