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

Catalog No：E-BC-K212-M

Specification：48T(22 samples)/96T(46 samples)

Measuring instrument：Microplate reader（660 nm）

Detection range：1.18-286.43 U/kg 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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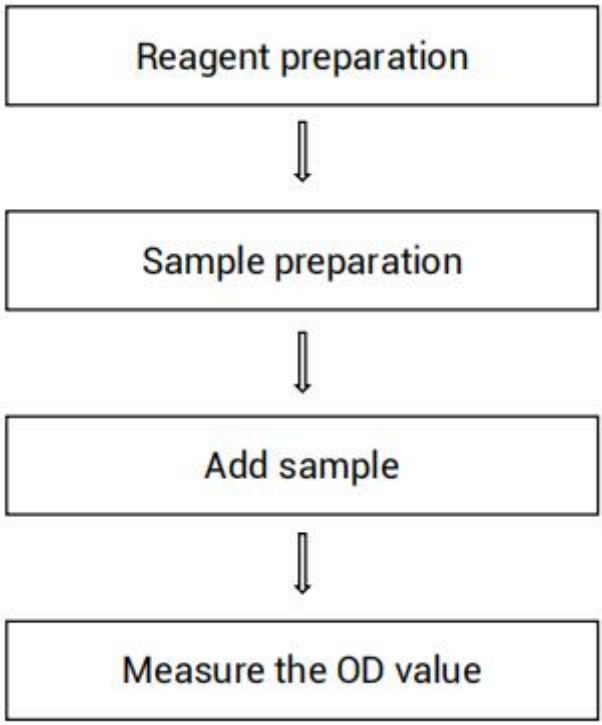
Assay summary



Intended use

This kit can be used to measure Ca^{2+}-ATPase activity in animal tissue 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(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	2-8℃, 12 months
Reagent 2	Activator A	1 mL × 1 vial	2 mL × 1 vial	2-8℃, 12 months
Reagent 3	Activator B	1 mL × 1 vial	2 mL × 1 vial	2-8℃, 12 months
Reagent 4	Substrate	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months
Reagent 5	Protein Precipitator	3 mL × 1 vial	6 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 Reagent	5 mL × 1 vial	10 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
	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:

Test tube, Vortex mixer, Incubator, Centrifuge, 100°C water bath, Microplate reader (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 5 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 7 days.

③ The preparation of chromogenic agent A working solution：
Dissolve one vial of chromogenic agent A with 5 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 7 days protected from light.

④ The preparation of chromogenic agent B working solution：
Dissolve one vial of chromogenic agent B with 5 mL of double distilled water in 90-100°C water bath, cool to room temperature before use. Store at 2-8°C for 7 days.

⑤ The preparation of phosphorus assay reagent：
For each well, prepare 200 μL of phosphorus assay reagent (mix well 80 μL of double distilled water, 40 μL of chromogenic agent A working solution, 40 μL of acid reagent and 40 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. Store at 2-8°C for 5 days protected from light.

⑥ The preparation of 0.5 mmol/L standard：
For each well, prepare 20 μL of 0.5 mmol/L standard (mix well 1 μL of 10 mmol/L standard and 19 μL of double distilled water). The 0.5 mmol/L standard should be prepared on spot. Store at 2-8°C for 7 days.

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).

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% Mouse liver tissue homogenate	1
10% Rat heart tissue homogenate	5-8
10% Rat lung tissue homogenate	5-8
10% Rat kidney 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.

③ To avoid external phosphorus contamination, be careful during the experiment.

Operating steps

Incubation reaction

① Non-enzyme tube: add 170 μL of buffer solution to the 1.5 mL EP tube.
Enzyme tube: add 170 μL of buffer solution to the 1.5 mL EP tube.

② Add 40 μL of activator A to the non-enzyme tube, and add 40 μL of activator B to the enzyme tube.

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

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

⑤ Incubate at 37℃ for 20 min.

⑥ Add 50 μL of protein precipitator to the non-enzyme tube and mix fully. Then add 200 μL of sample to the non-enzyme tube. 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.

Chromogenic reaction

① Blank well: add 20 μL of double distilled water to the corresponding wells.
Standard well: add 20 μL of 0.5 mmol/L standard to the corresponding wells.
Control well: add 20 μL of supernatant from non-enzyme tube to the corresponding wells.
Sample well: add 20 μL of the supernatant from enzyme tube to the corresponding wells.

② Add 200 μL of phosphorus assay reagent to each well.

③ Mix fully with microplate reader and incubate at 37℃ for 30 min. Measure the OD value of each well at 660 nm with microplate reader.

Calculation

The sample:

1. Tissue sample (Calculated by tissue protein):

Definition: The amount of Ca^{2+} -ATPase in 1 g tissue protein per 1 hour that decompose the ATP to produce 1 mmol inorganic phosphorus at 37°C is defined as 1 unit.

$$\text{Ca}^{2+}\text{ - ATP activity (U/gprot)} = (A_2 \div A_1) \times C \div T \times (V_1 \div V_2) \div C_{pr} \times f$$

2. Tissue sample (Calculated by tissue wet weight):

Definition: The amount of Ca^{2+} -ATPase in 1 kg wet weight per 1 hour that decompose the ATP to produce 1 mmol inorganic phosphorus at 37°C is defined as 1 unit.

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

[Note]

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

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

C: The concentration of standard, 0.5 mmol/L

T: The time of incubation reaction, 20 min=1/3 h

V_1: The total volume of incubation reaction, 0.5 mL.

V_2 The volume of sample, 0.2 mL.

V_3: The volume of normal saline homogenate, mL

m: The weight of tissue, g

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

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver 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)	5.60	85.40	195.00
%CV	6.5	6.1	5.4

Inter-assay Precision

Three rat liver 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)	5.60	85.40	195.00
%CV	7.6	8.2	8.2

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 107%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/kg wet weight)	25.8	103	242
Observed Conc. (U/kg wet weight)	28.4	111.2	249.3
Recovery rate (%)	110	108	103

Sensitivity

The analytical sensitivity of the assay is 1.18 U/kg 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 Π Example Analysis

Example analysis：

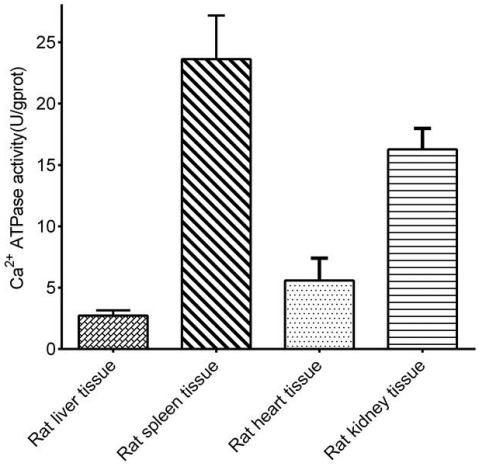
For 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation steps. The results are as follows:

the average OD value of the control is 0.199, the average OD value of the sample is 0.215, the average OD value of the standard is 0.296, the average OD value of the blank is 0.051, the concentration of protein in sample is 8.96 gprot/L, and the calculation result is:

Ca^{2+} -ATP activity (U/gprot)

= (0.215 - 0.199) ÷ (0.296 - 0.051) × 0.5 × 3 × (0.5 ÷ 0.2) ÷ 8.96 × 5= 0.13 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 8.96 gprot/L, dilute for 5 times), 10% rat spleen tissue homogenate (the concentration of protein is 5.80 gprot/L, dilute for 5 times), 10% rat heart tissue homogenate (the concentration of protein is 4.46 gprot/L, dilute for 5 times) and 10% rat kidney tissue homogenate (the concentration of protein is 7.79 gprot/L, 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.