Technical Manual

Ca^{2+}-ATPase Colorimetric Assay Kit

- **Catalogue Code: MAES0146**
- **Size: 100 Assays**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.8-41 U/g wet weight

Sensitivity:

0.8 U/g wet weight

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

ATPase exists on the membrane of tissue cells and organelles. It is a kind of protease on the biological membrane which plays an important role in material transport, energy conversion and information transmission. Ca^{2+} which participates in the regulation of different enzyme systems and cell activities plays many important roles in cells. The flow of Ca^{2+} depends on the Ca^{2+} -ATPase on the cell membrane, and Ca^{2+} -ATPase consumes ATP to generate the energy needed for ion transport.

3. Intended Use

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

4. 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. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	20 mL × 1 vial	2-8°C, 6 months
Accelerator A	2 mL × 2 vials	2-8°C, 6 months
Accelerator B	2 mL × 2 vials	2-8°C, 6 months
Substrate	Lyophilized × 1 vial	2-8°C, 6 months
Protein Precipitator	10 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent A	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Acid Agent	50 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent B	Lyophilized × 2 vials	2-8°C, 6 months
Standard Solution (10 mmol/L)	2 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (660nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

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.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution:** Dissolve a vial of substrate lyophilized with 10 mL double distilled water. The prepared solution can be stored at 2-8°C for a week.

3. Preparation of **chromogenic agent A working solution:** Dissolve a vial of chromogenic agent A lyophilized with 25 mL double distilled water. The prepared solution can be stored at 2-8°C with avoid direct sunlight for a week.

4. Preparation of **chromogenic agent B working solution:** Dissolve a vial of chromogenic agent B lyophilized with 25 mL of double distilled water at 90-100°C. The prepared solution can be stored at 2-8°C for a week.

5. Preparation of **phosphorus assay reagent:** Mix double distilled water, chromogenic agent A working solution, acid agent, chromogenic agent B working solution at a ratio of 2:1:1:1. Prepared solution should be pale yellow. If it is colorless or blue, it should be invalid or phosphorus pollution. Prepare the fresh phosphorus assay reagent before use and the prepared should be with avoid direct sunlight.

6. Preparation of **standard (0.5 µmol/mL):** Dilute standard solution (10 mmol/L) with double distilled water for 20 times. The prepared solution can be stored at 2-8°C with for a week.

8. Sample Preparation

Tissue sample:
Take 0.02-1g fresh tissue to wash with normal saline (0.9% NaCl) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.8-41 U/g wet weight).

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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 660nm

10. Operation Steps

Enzymatic reaction

1. **Control tube:** take 170 μL of buffer solution to 1.5 mL EP tube.
   Sample tube: take 170 μL of buffer solution to 1.5 mL EP tube.

2. Add 40 μL of accelerator A to **control tube**.

3. Add 40 μL of accelerator B to **sample tube**.

4. Add 40 μL of substrate working solution to **each tube**.

5. Add 200 μL of sample to **sample tube** and mix fully with vortex mixer.

6. Incubate at 37°C for 10 min.

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

8. Add 50 μL of protein precipitator to sample tube.

9. Mix fully and centrifuge at 2000 g for 10 min, take supernatant of each tube for detection.

Color reaction

1. **Standard tube:** take 200 μL of 0.5 μmol/mL standard to 5 mL EP tube
   Blank tube: take 200 μL of double distilled water to 5 mL EP tube
   Control tube: take 200 μL of supernatant from corresponding control tube to 5 mL EP tube.
   Sample tube: take 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 fully, incubate at 37°C 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.

Operation Table (Enzymatic reaction)

	Control tube	Sample tube
Buffer solution (μL)	170	170
Accelerator A (μL)	40	
Accelerator B (μL)		40
Substrate working solution (μL)	40	40
Sample (μL)		200
Mix fully and incubate at 37°C for 10 min.		
Protein precipitator (μL)	50	50
Sample (μL)	200	
Mix fully and centrifuge at 2000 g for 10 min, take supernatant of each tube for detection.		

Operation Table (Color reaction)

	Standard tube	Blank tube	Control tube	Sample tube
0.5 μmol/mL Standard (μL)	200			
Double distilled water (μL)		200		
Supernatant of control tube (μL)			200	
Supernatant of sample tube (μL)				200
Phosphorus assay reagent (μL)	2000	2000	2000	2000
Mix fully, incubate at 37°C for 10 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.				

11. Calculations

1. For tissue (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°C is defined as 1 ATPase activity unit.

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

2. For tissue (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.

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

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 enzymatic reaction, 10 min = 1/6 h

V_1: the total volume of incubation reaction, 500 μL

V_2: the volume of sample added to the reaction, 200 μL

V_3: the volume of normal saline homogenate

m: the weight of tissue

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

f: Dilution factor of sample before tested

12. Performance Characteristics

Detection Range	0.8-41 U/g wet weight
Sensitivity	0.8 U/g wet weight
Average inter-assay CV (%)	8.8
Average intra-assay CV (%)	4.1

Analysis

Take 10% mouse kidney tissue homogenate, dilute for 5 times, carry the assay according to the operation table.

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+} ATPase activity (U/mgprot)

= (0.323-0.152) ÷ (0.405-0.001) × 0.5 × 6× 500 ÷ 200 × 5 ÷ 6.69 = 2.37 U/mgprot

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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