TECHNICAL MANUAL

Ca2+-ATPase Activity Assay Kit

- **SKU CODE:** MAES0213
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Incubation reaction
- Chromogenic reaction
- Measure optical density at 660 nm

2. Intended use

This kit can be used to measure Ca2+-ATPase activity in animal tissue samples.

3. 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, Ca2+-ATPase activity is inhibited, while in the sample system, Ca2+-ATPase activity is not inhibited. The difference in inorganic phosphorus content between the sample and the control represents the inorganic phosphorus produced by Ca2+-ATPase during the incubation time. The activity of Ca2+-ATPase is determined by measuring inorganic phosphorus production.

4. 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°C, 12 months
Reagent 2	Activator A	1 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months
Reagent 3	Activator B	1 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months
Reagent 4	Substrate	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 5	Protein Precipitator	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 7	Acid Reagent	5 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 8	Chromogenic Agent B	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months
Reagent 9	10 mmol/L Standard Solution	2 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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. **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. **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. **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. **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.

7. 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°C.

4. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

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.

8. 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.

9. 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°C 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°C for 30 min.

Measure the OD value of each well at 660 nm with microplate reader.

10. Calculation

Tissue sample (Calculated by tissue protein):

Definition: The amount of Ca2+-ATPase in 1 g tissue protein per 1 hour that decomposes the ATP to produce 1 mmol inorganic phosphorus at 37°C is defined as 1 unit.

Ca2+-ATPase activity (U/g_{prot}) = $(A_2 \div A_1) \times C \div T \times (V_1 \div V_2) \div C_{pr} \times f$

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

Definition: The amount of Ca2+-ATPase in 1 kg wet weight per 1 hour that decomposes the ATP to produce 1 mmol inorganic phosphorus at 37°C is defined as 1 unit.

Ca2+-ATPase 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, g_{prot}/L

f: Dilution factor of sample before test

11. Appendix I Performance Characteristics

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

Three samples of high concentration, middle concentration and low concentration were tested with each concentration assayed 6 times in parallel to obtain an 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.

12. Appendix II Example Analysis

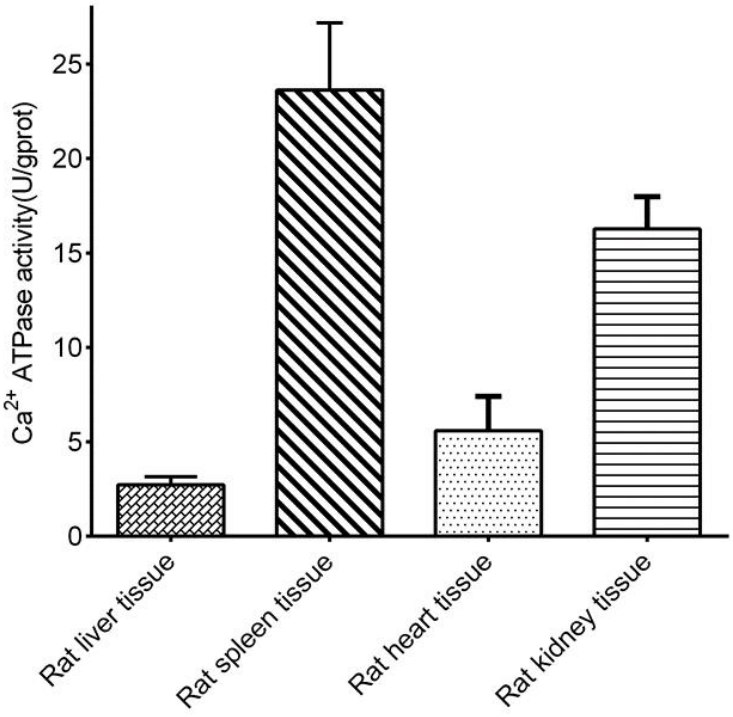
Example analysis:

For 10% rat liver tissue homogenate, diluted 5 times, and carried out 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 g_{prot}/L, and the calculation result is:

Ca2+-ATPase activity (U/g_{prot}) = (0.215 - 0.199) ÷ (0.296 - 0.051) × 0.5 × 3 × (0.5 ÷ 0.2) ÷ 8.96 × 5 = 0.13 U/g_{prot}

Detect 10% rat liver tissue homogenate (the concentration of protein is 8.96 g_{prot}/L, diluted 5 times), 10% rat spleen tissue homogenate (the concentration of protein is 5.80 g_{prot}/L, diluted 5 times), 10% rat heart tissue homogenate (the concentration of protein is 4.46 g_{prot}/L, diluted 5 times) and 10% rat kidney tissue homogenate (the concentration of protein is 7.79 g_{prot}/L, diluted 5 times) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.