TECHNICAL MANUAL

Na+k+-ATPase Activity Assay Kit

- **SKU CODE:** MAES0188
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Enzymatic reaction
- Chromogenic reaction
- Calculate results

2. Intended use

This kit can be used to measure Na+K+-ATPase activity in serum, plasma and animal tissue samples.

3. Detection principle

Na+K+-ATPase decomposes ATP to produce ADP and phosphorus. The activity of Na+K+-ATPase is calculated by measuring the phosphorus content.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	6 mL × 1 vial	2-8°C, 12 months
Reagent 2	Accelerant A	5 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate	Powder × 1 vial	2-8°C, 12 months
Reagent 4	Accelerant B	1.5 mL × 1 vial	2-8°C, 12 months
Reagent 5	Protein Precipitator	1.5 mL × 2 vials	2-8°C, 12 months
Reagent 6	Powder A	Powder × 1 vial	2-8°C, 12 months, protect from light
Reagent 7	Powder B	Powder × 1 vial	2-8°C, 12 months, protect from light
Reagent 8	Acid Reagent	5 mL × 1 vial	2-8°C, 12 months
Reagent 9	10 µmol/mL Pi Standard	2 mL × 1 vial	2-8°C, 12 months

Item	Component	Size (96 T)	Storage
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Centrifuge, Incubator, Microplate reader (640-680nm, optimum wavelength: 660 nm).

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Substrate working solution preparation:** Dissolve one vial of substrate with 5 mL double distilled water. Store at -20°C for 7 days.
3. **Powder A working solution preparation:** Dissolve one vial of powder A with 5 mL double distilled water. Store at 2-8°C for 7 days protected from light.
4. **Powder B working solution preparation:** Dissolve one vial of powder B with 5 mL double distilled water. Store at 2-8°C for 7 days protected from light.
5. **Chromogenic working solution preparation:** For each well, prepare 200 µL of chromogenic working solution by mixing 80 µL of double distilled water, 40 µL of powder A working solution, 40 µL of powder B working solution and 40 µL of acid reagent. The chromogenic working solution should be prepared fresh and used the same day.
6. **Standard curve preparation:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 µmol/mL phosphorus standard solution with normal saline to the following serial concentrations: 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 µmol/mL.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month.

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 10,000×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 (MAES0177).

Recommended dilution factors for different samples (for reference only):

- Rat plasma: 1

- 10% Rat liver tissue homogenate: 6-10

- 10% Rat spleen tissue homogenate: 4-8

- 10% Rat heart tissue homogenate: 4-8

- 10% Mouse brain tissue homogenate: 4-8

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

8. The key points of the assay

1. Prevent phosphorus contamination during operation. The containers and test tubes involved in the detection process must be disposed of strictly without a trace of phosphorus. It is better to use disposable EP tubes or new glass tubes to avoid phosphorus contamination, which is the key for success.
2. The prepared chromogenic working solution should be pale yellow. If it is other colors, it should be invalid or phosphorus contaminated and needs to be re-prepared.
3. When ΔA_660 of sample is more than 0.160, please dilute the sample and test again.

9. Operating steps

Enzymatic reaction:

1. Control tube: Take 65 µL of buffer solution to 1.5 mL EP tube.

Sample tube: Take 45 µL of buffer solution to 1.5 mL EP tube.

2. Add 40 µL of accelerant A and 20 µL of substrate working solution to each tube.

3. Add 20 µL of accelerant B and 100 µL of sample to sample tube.

4. Mix fully for 3 s and incubate at 37°C for 10 min.

5. Add 25 µL of protein precipitator to each tube.

6. Add 100 µL of sample to control tube.

7. Mix fully for 3 s and centrifuge at 8,000×g for 10 min. Take supernatant of each tube for chromogenic reaction.

Chromogenic reaction:

1. Standard well: Take 20 µL of standard with different concentration to corresponding standard well.

Control well: Take 20 µL of supernatant from corresponding control tube in enzymatic reaction step.

Sample well: Take 20 µL of supernatant from corresponding sample tube in enzymatic reaction step.

2. Add 200 µL of chromogenic working solution to each well.

3. Mix fully for 10 s with microplate reader, incubate at 37°C for 15 min and measure the OD value of each well at 660 nm.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) and other liquid sample:

Definition: 1 µmol of inorganic phosphorus produced through the decomposition of ATP by Na+K+-ATPase of 1 mL of serum (plasma) per hour is defined as 1 unit.

Na+K+-ATPase activity (µmol Pi/mL/hour) = $(\Delta A\_660 - b) \div a \times V_1 \div V_2 \div t \times f$

2. Tissue sample:

Definition: 1 µmol of inorganic phosphorus produced through the decomposition of ATP by Na+K+-ATPase of 1 mg of tissue protein per hour is defined as 1 unit.

Na+K+-ATPase activity (μmol Pi/mgprot/hour) = (ΔA_660 - b) ÷ a × V_1 ÷ (C_{pr} × V_2) ÷ t × f

[Note]

ΔA_660: OD_{Sample} – $OD_{Control}$.

V_1: The total volume of reaction system (0.25 mL).

V_2: The volume of added sample (0.1 mL).

t: The time of enzymatic reaction (1/6 h).

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

f: Dilution factor of sample before tested.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol Pi/mL/hour)	0.85	1.35	2.80
%CV	2.5	2.2	1.9

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol Pi/mL/hour)	0.85	1.35	2.80
%CV	1.9	2.4	2.3

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 104%.

Recovery

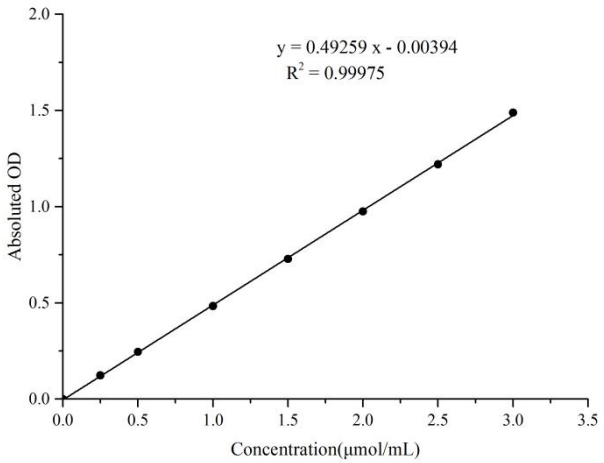
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/mL)	0.35	1.05	2.3
Observed Conc. (µmol/mL)	0.4	1.1	2.4
Recovery rate (%)	101	106	105

Sensitivity

The analytical sensitivity of the assay is 0.11 µmol Pi/mL/hour. 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.

Standard curve

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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/mL)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.037	0.038	0.038	0
0.25	0.161	0.161	0.161	0.123
0.5	0.283	0.283	0.283	0.245
1.0	0.522	0.520	0.521	0.483
1.5	0.766	0.765	0.766	0.728
2.0	1.021	1.004	1.012	0.974
2.5	1.259	1.255	1.257	1.219
3.0	1.535	1.517	1.526	1.488

12. Appendix II Example Analysis

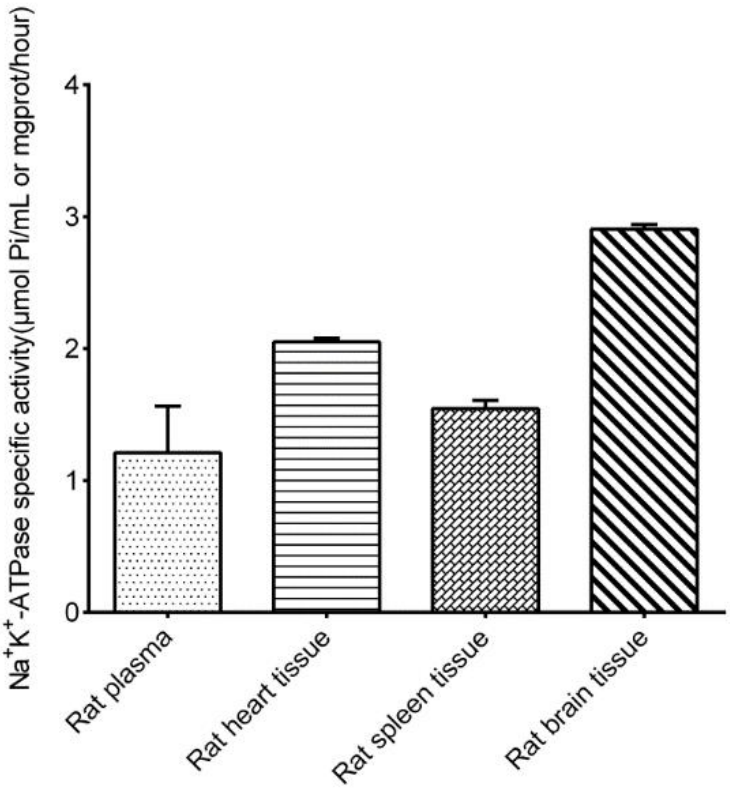
Example analysis:

For rat heart tissue, take the supernatant of freshly prepared 10% rat heart sample, dilute with normal saline 4 times and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.4926x – 0.0039, the average OD value of the sample is 0.267, the average OD value of the control is 0.165, the concentration of protein in sample is 6.23 mgprot/mL, and the calculation result is:

Na+K+-ATPase activity (μmol Pi/mgprot/hour) = (0.267 - 0.165 + 0.0039) ÷ 0.4926 × 0.25 ÷ (6.23 ÷ 4 × 0.1) × 6 = 2.07 μmol Pi/mgprot/hour

Detection of rat plasma, 10% rat heart tissue homogenate (protein concentration is 6.23 mgprot/mL, diluted 4 times), 10% rat spleen tissue homogenate (protein concentration is 9.31 mgprot/mL, diluted 4 times) and 10% rat brain tissue homogenate (protein concentration is 6.52 mgprot/mL, diluted 4 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.