Technical Manual

Na^+k^+-ATPase Activity Assay Kit

- **Catalogue Code: MAES0188**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.42-4.99 µmol Pi/mL/hour

Sensitivity:

0.11 µmol Pi/mL/hour

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

Na^+K^+-ATPase (EC3.6.1.37) is a glycoprotein located on the cell membrane, which is closely related to the decomposition of ATP and the transport of sodium and potassium ions inside and outside the cell. It plays a vital role in maintaining the normal functional state of various cells and the normal physiological function of the human body.

3. Intended Use

The kit is used for the determination of Na^+K^+-ATPase activity in serum, plasma, whole blood and animal tissue samples.

4. Detection Principle

Na^+K^+-ATPase decomposes ATP to produce ADP and Phosphorus, and calculates the activity of Na+K+-ATPase by measuring the content of phosphorus.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	6 mL × 1 vial	2-8°C, 6 months
Accelerant A	5 mL × 1 vial	2-8°C, 6 months
Substrate	Lyophilized × 1 vial	2-8°C, 6 months
Accelerant B	1.5 mL × 1 vial	2-8°C, 6 months
Protein Precipitator	1.5 mL × 2 vials	2-8°C, 6 months
Powder A	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Powder B	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Acid Reagent	5 mL × 1 vial	2-8°C, 6 months
Pi Standard (10 μmol/mL)	2 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- incubator
- 37°C water bath
- Vortex mixer
- centrifuge
- Microplate reader (640-680 nm)
- 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. The tubes used in assay must be disposed strictly without a trace of phosphorus. It is better to use disposable tubes or new tubes to avoid pollution of phosphorus which is the key for success.

2. When ΔA_{660} of sample is more than 0.160, dilute the sample and test again.

7. Reagent Preparation:

1. Bring all the reagents to room temperature before use.

2. Preparation of **Substrate working solution:** Dissolve a vial of powder with 5 mL double distilled water. The prepared solution can be stored at -20°C for a week.

3. Preparation of **Powder A working solution:** Dissolve a vial of powder A with 5 mL double distilled water. The prepared solution can be stored at 2-8°and avoid direct sunlight for a week.

4. Preparation of **Powder B working solution:** Dissolve a vial of powder B with 5 mL double distilled water. The prepared solution can be stored at 2-8°C and avoid direct sunlight for a week.

5. Preparation of **chromogenic agent working solution:** Mix double distilled water, powder A working solution, powder B working solution, acid Reagent at a ratio of 2:1:1:1. Prepare the fresh solution before use and only available on the same day.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Blood sample:
Collect the fresh blood to the test tube containing anticoagulant, mix gently. And the sample can be stored at 2-8°C for 1-2 days.

4. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water 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 and preserve on ice before 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 (0.42-4.99 μmol Pi/mL/hour).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 660 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1'	S1	S9'	S9	S17'	S17	S25'	S25	S33'	S33
B	B	B	S2'	S2	S10'	S10	S18'	S18	S26'	S26	S34'	S34
C	C	C	S3'	S3	S11'	S11	S19'	S19	S27'	S27	S35'	S35
D	D	D	S4'	S4	S12'	S12	S20'	S20	S28'	S28	S36'	S36
E	E	E	S5'	S5	S13'	S13	S21'	S21	S29'	S29	S37'	S37
F	F	F	S6'	S6	S14'	S14	S22'	S22	S30'	S30	S38'	S38
G	G	G	S7'	S7	S15'	S15	S23'	S23	S31'	S31	S39'	S39
H	H	H	S8'	S8	S16'	S16	S24'	S24	S32'	S32	S40'	S40

Note: A-H, standard wells; S1'-S40', control wells; S1-S40, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 10 μmol/mL phosphorus standard solution with normal saline to a serial concentration. The recommended dilution gradient is as follows: 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 μmol/mL.

The measurement of samples

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 100μL 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 8000 g for 10 min, take supernatant of each tube for color assay.

Color 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 agent 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.

Operation Table

Enzymatic reaction

	Control tube	Sample tube
Buffer Solution (μL)	65	45
Accelerant A (μL)	40	
Substrate working solution (μL)	20	20
Accelerant B (μL)		20
Sample (μL)		100
Mix fully and incubate at 37°C for 10 min.		
Protein Precipitator (μL)	25	25
Sample (μL)	100	
Mix fully with the vortex mixer and centrifuge at 8000 g for 10 min, take supernatant of each tube for color assay.		

Color reaction

	Standard well	Control well	Sample well
Standard with different concentration (μL)	20		
Supernatant (μL)		20	20
chromogenic agent working solution (μL)	200	200	200
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.			

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

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

2. Tissue sample:

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

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0);
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve;
Δ_{A660}: 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.

12. Performance Characteristics

Detection Range	0.42-4.99 μmol Pi/mL/hour
Sensitivity	0.11 μmol Pi/mL/hour
Average recovery rate (%)	104
Average inter-assay CV (%)	2.2
Average intra-assay CV (%)	2.2

Analysis

For rat heart tissue, take the supernatant of fresh prepared 10% rat heart sample, dilute with normal saline for 4 times and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.4926 x – 0.0039, the average OD value of the sample is 0.267, the average OD value of the blank is 0.165, the concentration of protein in sample is 6.23 mgprot/mL.

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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