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

Catalog No：E-BC-K539-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（640-680 nm）

Detection range：0.42-4.99 μmol Pi/mL/hour

Elabscience® Na^+K^+-ATPase Activity Assay Kit

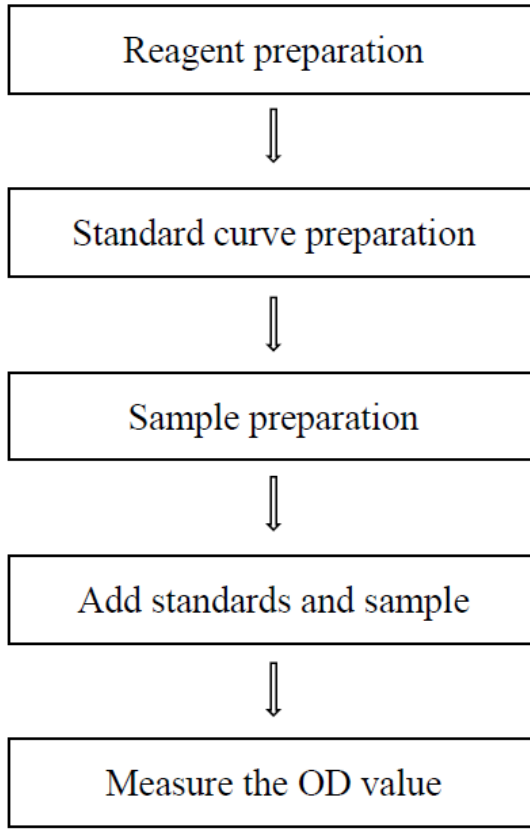
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	6 mL × 1 vial	2-8 ℃, 12 months
Reagent 2	Accelerant A	5 mL × 1 vial	2-8 ℃, 12 months
Reagent 3	Substrate	Powder × 1 vial	2-8 ℃, 12 months
Reagent 4	Accelerant B	1.5 mL × 1 vial	2-8 ℃, 12 months
Reagent 5	Protein Precipitator	1.5 mL × 2 vials	2-8 ℃, 12 months
Reagent 6	Powder A	Powder × 1 vial	2-8 ℃, 12 months, shading light
Reagent 7	Powder B	Powder × 1 vial	2-8 ℃, 12 months, shading light
Reagent 8	Acid Reagent	5 mL × 1 vial	2-8 ℃, 12 months
Reagent 9	10 μmol/mL Pi Standard	2 mL × 1 vial	2-8 ℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution:
Dissolve a vial of substrate with 5 mL double distilled water. Store at -20 ℃ for 7 days.

③ The preparation of powder A working solution:
Dissolve a vial of powder A with 5 mL double distilled water. Store at 2-8 ℃ for 7 days protected from light.

④ The preparation of powder B working solution:
Dissolve a vial of powder B with 5 mL double distilled water. Store at 2-8 ℃ for 7 days protected from light.

⑤ The preparation of chromogenic working solution:
For each well, prepare 200 μL of chromogenic working solution (mix well 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 on spot and used up on the same day.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**0.25**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**
10 μmol/mL Standard Solution (μL)	0	25	50	100	150	200	250	300
Normal saline (μL)	1000	975	950	900	850	800	750	700

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a 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℃.

④ Centrifuge at 10000×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 (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
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 the dilution of other sample types, please do pretest to confirm the dilution factor

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 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 contamination and it needs to be re-prepared.

③ When ΔA_{660} of sample is more than 0.160, please dilute the sample and test again.

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 ℃ 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 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 ℃ for 15 min and measure the OD value of each well at 660 nm.

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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.

$$\underset{(\mu mol\ Pi/mL/hour)}{Na^+K^+\text{-ATPase activity}} = (\Delta A_{660} - b) \div a \times V_1 \div V_2 \div t \times f$$

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.

$$\underset{(\mu mol\ Pi/mgprot/hour)}{Na^+K^+\text{-ATPase activity}} = (\Delta A_{660} - b) \div a \times V_1 \div (C_{pr} \times V_2) \div t \times f$$

[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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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 (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.

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

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 104%.

	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

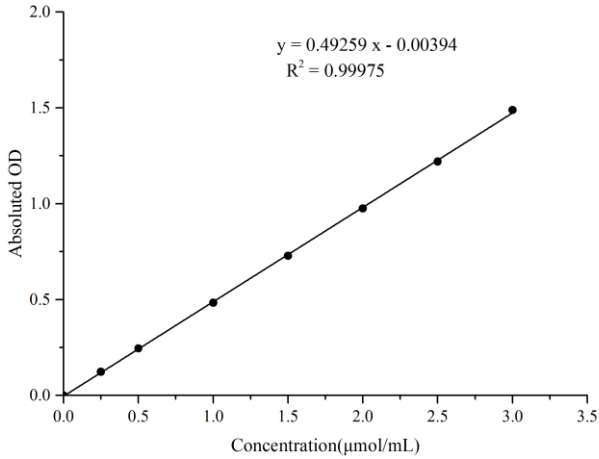
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

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



Appendix II Example Analysis

Example analysis:

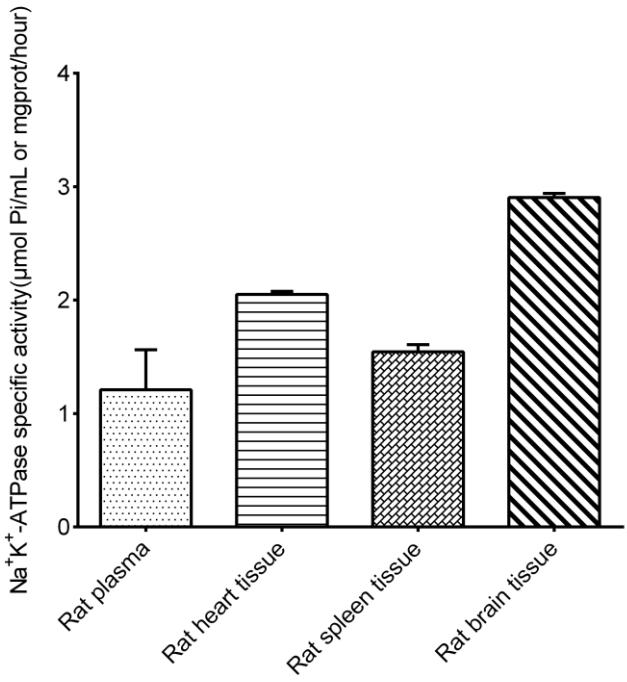
For rat heart tissue, take the supernatant of fresh prepared 10% rat heart sample, dilute with normal saline for 4 times and carry the assay according to the operation steps. The results are as follows:

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

$$\underset{(\mu mol\ Pi/mgprot/hour)}{Na^+K^+\text{-ATPase activity}} = (0.267 - 0.165 + 0.0039) \div 0.4926 \times 0.25 \div (6.23 \div 4 \times 0.1) \times 6$$

$$= 2.07\ \mu mol\ Pi/mgprot/hour$$

Detect rat plasma, 10% rat heart tissue homogenate (the concentration of protein is 6.23 mgprotm/L, dilute for 4 times), 10% rat spleen tissue homogenate (the concentration of protein is 9.31 mgprot/mL, dilute for 4 times) and 10% rat brain tissue homogenate (the concentration of protein is 6.52 mgprot/mL, dilute for 4 times) according to the protocol, the result is as follows:


Na+K+-ATPase specific activity(μmol Pi/mL or mgprot/hour)
0
1
2
3
4
Rat plasma
Rat heart tissue
Rat spleen tissue
Rat brain tissue

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.