PRODUCT INFORMATION & MANUAL

Na+/K+ ATPase Activity Assay Kit (Colorimetric)

NBP3-25857

For research use only.
Not for diagnostic or therapeutic procedures.

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Na+/K+ ATPase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25857

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.11 μmol Pi/mL/hour

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

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 2.2

Verage recovery rate (%): 104

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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	Specification	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.			

▲Materials prepared by users

Instruments

Microplate reader (640-680 nm), micropipettor, tubes, vortex mixer, incubator, 37℃ water bath, centrifuge.

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

Precautions

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

The key points of the assay

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, please dilute the sample and test again

Pre-assay preparation

▲Reagent preparation

1. Bring all the reagents to room temperature before use.
2. Preparation of reagent 3 working solution:
   Dissolve a vial of powder with 5 mL double distilled water. The prepared solution can be stored at -20℃ for a week.
3. Preparation of reagent 6 working solution:
   Dissolve a vial of powder with 5 mL double distilled water. The prepared solution can be stored at 2-8℃ with shading light for a week.
4. Preparation of reagent 7 working solution:
   Dissolve a vial of powder with 5 mL double distilled water. The prepared solution can be stored at 2-8℃ with shading light for a week.
5. Preparation of chromogenic agent working solution:
   Mix double distilled water, reagent 6 working solution, reagent 7 working solution, reagent 8 at a ratio of 2:1:1:1. Prepare the fresh solution before use and only available on the same day.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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).

Assay protocol

▲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.

▲Detailed operating steps

1. The measurement 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. Reference is as follows:

Number	Standard concentrations (μmol/mL)	10 μmol/mL Standard(μL)	Normal saline (μL)
A	0	0	1000
B	0.25	25	975
C	0.5	50	950
D	1.0	100	900
E	1.5	150	850
F	2.0	200	800
G	2.5	250	750
H	3.0	300	700

2. The measurement of samples

1) Enzymatic reaction

(1) Control tube: take 65 μL of reagent 1 to 1.5 mL EP tube.
Sample tube: take 45 μL of reagent 1 to 1.5 mL EP tube.

(2) Add 40 μL of reagent 2 and 20 μL of reagent 3 working solution to each tube.

(3) Add 20 μL of reagent 4 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 reagent 5 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.

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

▲Summary operation table

1) Enzymatic reaction

	Control tube	Sample tube
Reagent 1 (μL)	65	45
Reagent 2 (μL)	40	40
Reagent 3 working solution (μL)	20	20
Reagent 4 (μL)		20
Sample (μL)		100
Mix fully and incubate at 37℃ for 10 min.		
Reagent 5 (μL)	25	25
Sample (μL)	100	
Mix fully and centrifuge at 8000 g for 10 min, take supernatant of each tube for color assay.		

2) 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, incubate at 37℃ for 15 min and measure the OD value at 660 nm.			

▲Calculation

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. unit.

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.

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 ATPase of 1 mg of tissue protein per hour is defined as 1 ATPase activity unit.

Na^+K^+-ATPase activity (μmol Pi/mgprot/hour) = $(\Delta A_{660} - b) \div a \times V_1 \div$

$(C_{pr} \times V_2) \div t \times f$

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;

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 Data

▲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 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, and the calculation result is:

Na^+K^+-ATPase activity (μmol Pi/mgprot/hour)
=(0.276-0.165+0.0039)÷0.4926×0.25÷(6.23×0.1) ÷ (1/6)×4
= 2.07 μmol Pi/mgprot/hour

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum (plasma)

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. The serum must be centrifuged before test if it is turbid. Place the serum on ice for detection.

▲Whole blood sample

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

▲Tissue sample

Take 0.02-1 g tissue sample, wash with normal saline (0.9% NaCl) at 2-8℃. Absorb the water with filter paper and weigh. Then add 9 times the volume of normal saline according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)