PRODUCT INFORMATION & MANUAL

Calcium ATPase Activity Assay Kit (Colorimetric)

NBP3-25909

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

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Ca^{2+}-ATPase Activity Assay Kit

Catalog No: NBP3-25909

Method: Colorimetric method

Specification: 100 Assays (Can detect 48 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.8 U/g wet weight

Detection range: 0.8-41 U/g wet weight

Average intra-assay CV (%): 4.1

Average inter-assay CV (%): 8.8

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

This kit can be used for detection of Ca^{2+}-ATPase activity in animal tissue samples.

▲Background

ATPase exists on the membrane of tissue cells and organelles. It is a kind of protease on the biological membrane which plays an important role in material transport, energy conversion and information transmission. Ca^{2+}-ATPase which participates in the regulation of different enzyme systems and cell activities plays many important roles in cells. The flow of Ca^{2+}-ATPase depends on the Ca^{2+}-ATPase on the cell membrane, and Ca^{2+}-ATPase consumes ATP to generate the energy needed for ion transport.

▲Detection principle

ATPase can decompose ATP to produce ADP and inorganic phosphorus. The activity of ATPase can be expressed by measuring the production amount of inorganic phosphorus in unit time. In the control system, Ca^{2+} -ATPase activity was inhibited, while in the sample system, Ca^{2+} -ATPase activity was not inhibited. The difference of inorganic phosphorus content between the sample and the control was the inorganic phosphorus produced by Ca^{2+} -ATPase during the incubation time. The activity of Ca^{2+} -ATPase was determined by inorganic phosphorus production.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Accelerator A	2 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Accelerator B	2 mL × 2 vials	2-8℃ , 12 months
Reagent 4	Substrate	Powder × 1 vial	2-8℃ , 12 months
Reagent 5	Protein Precipitator	10 mL × 1 vial	2-8℃ , 12 months
Reagent 6	Chromogenic Agent A	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 7	Acid Agent	50 mL × 1 vial	2-8℃ , 12 months
Reagent 8	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 12 months
Reagent 9	10 mmol/L Standard Solution	2 mL × 1 vial	2-8℃ , 12 months
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

Spectrophotometer (660 nm), Test tube, Micropipettor, Vortex mixer, Incubator, Centrifuge

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. 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.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 4 working solution:
   Dissolve a vial of powder with 10 mL double distilled water. The prepared solution can be stored at 2-8℃ for a week.
3. Preparation of reagent 6 working solution:
   Dissolve a vial of powder with 25 mL double distilled water. The prepared solution can be stored at 2-8℃ with shading light for a week.
4. Preparation of reagent 8 working solution:
   Dissolve a vial of powder with 25 mL of double distilled water at 90-100°C. The prepared solution can be stored at 2-8℃ for a week.
5. Preparation of phosphorus assay reagent:
   Mix double distilled water, reagent 6 working solution, reagent 7, reagent 8 working solution at a ratio of 2:1:1:1. Prepared solution should be pale yellow. If it is colorless or blue, it should be invalid or phosphorus pollution. Prepare the fresh phosphorus assay reagent before use and the prepared should be with shading light.
6. Preparation of 0.5 μmol/mL standard:
   Dilute the reagent 9 with double distilled water for 20 times. The prepared solution can be stored at 2-8℃ with for a week.

▲Sample preparation

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 (E-BC-K318-M)

▲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 ((0.8-41 U/g wet weight).

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% Rat heart tissue homogenate	5-8
10% Rat kidney tissue homogenate	5-8
10% Mouse liver tissue homogenate	1
10% Rat lung tissue homogenate	5-8
10% Rat brain tissue homogenate	2-3

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Detailed operating steps

1. Enzymatic reaction

(1) Sample tube: take 170 μL of reagent 1 to 1.5 mL EP tube.
Control tube: take 170 μL of reagent 1 to 1.5 mL EP tube.

(2) Add 40 μL of reagent 2 to control tube.
Add 40 μL of reagent 3 to sample tube.

(3) Add 40 μL of reagent 4 working solution to each tube.

(4) Add 200 μL of sample to sample tube and mix fully with vortex mixer.

(5) Incubate at 37℃ for 10 min.

(6) Add 50 μL of reagent 5 to control tube, mix fully and add 200 μL of sample.
Add 50 μL of reagent 5 to sample tube.

(7) Mix fully and centrifuge at 2000 g for 10 min, take supernatant of each tube for detection.

2. Color reaction

(1) Standard tube: take 200 μL of 0.5 μmol/mL standard to 5 mL EP tube
Blank tube: take 200 μL of double distilled water to 5 mL EP tube
Control tube: take 200 μL of supernatant from corresponding control tube to 5 mL EP tube.
Sample tube: take 200 μL of supernatant from corresponding sample tube to 5 mL EP tube.

(2) Add 2000 μL of phosphorus assay reagent to each tube.

(3) Mix fully, incubate at 37℃ for 30 min. Set the spectrophotometer to zero with distilled water and measure the OD of each tube at 660 nm with 1 cm optical path quartz cuvette.

▲Summary operation table

1. Enzymatic reaction

	Control tube	Sample tube
Reagent 1 (μL)	170	170
Reagent 2 (μL)	40	
Reagent 3 (μL)		40
Reagent 4 working solution (μL)	40	40
Sample (μL)		200
Mix fully and incubate at 37℃ for 10 min.		
Reagent 5 (μL)	50	50
Sample (μL)	200	
Mix fully and centrifuge for 10 min, take supernatant of each tube for detection.		

2. Color reaction

	Standard tube	Blank tube	Control tube	Sample tube
0.5 μmol/mL Standard (μL)	200			
Double distilled water (μL)		200		
Supernatant of control tube (μL)			200	
Supernatant of sample tube (μL)				200
Phosphorus assay reagent (μL)	2000	2000	2000	2000
Mix fully, incubate at 37℃ for 10 min. Set the spectrophotometer to zero with distilled water and measure the OD at 660 nm with.				

▲Calculation

1. For tissue (tissue protein):

Unit definition: 1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 1 mg of tissue protein per hour at 37℃ is defined as 1 ATPase activity unit.

$$Ca^{2+}\text{-ATPase activity (U/mgprot)} = A_2 \div A_1 \times C \div t \times V_1 \div V_2 \div C_{pr} \times f$$

2. For tissue (wet weight):

Unit definition: 1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 1 g of wet weight per hour at 37℃ is defined as 1 ATPase activity unit.

$$Ca^{2+}\text{-ATPase activity (U/g wet weight)} = A_2 \div A_1 \times C \div t \times V_1 \div V_2 \div (m \div V_3) \times f$$

Note:

A_2: $OD_{Sample} - OD_{Control}$;

A_1: $OD_{Standard} - OD_{Blank}$;

C: the concentration of standard, 0.5 μmol/mL;

t: the time of enzymatic reaction, 10 min = 1/6 h;

V_1: the total volume of incubation reaction, 500 μL;

V_2: the volume of sample added to the reaction, 200 μL;

V_3: the volume of normal saline homogenate;

m: the weight of tissue;

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

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

Take 10% mouse kidney tissue homogenate, dilute for 5 times and carry the assay according to the operation table. The results are as follows:

The OD value of the control tube is 0.152, the OD value of the sample tube is 0.323, the OD value of the standard tube is 0.405, the OD value of the blank tube is 0.001, the concentration of protein in sample is 6.69 mgprot/mL, and the result is:

Ca^{2+}-ATPase activity (U/mgprot)

= (0.323-0.152)÷(0.405-0.001)× 0.5×6×500÷200×5÷6.69 = 2.37 U/mgprot