PRODUCT INFORMATION & MANUAL

Calcium ATPase Activity Assay Kit (Colorimetric)

NBP3-25902

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

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Ca^{2+}-ATPase Activity Assay Kit

Catalog No: NBP3-25902

Method: Colorimetric method

Specification: 96T (Can detect 46 samples without duplication)

Instrument: Microplate reader

Sensitivity: 1.18 U/kg wet weight

Detection range: 1.18-286.43 U/kg wet weight

Average intra-assay CV (%): 6.0

Average inter-assay CV (%): 8.0

Average recovery rate (%): 107

- ▲ 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 measure 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+} which participates in the regulation of different enzyme systems and cell activities plays many important roles in cells. The flow of Ca^{2+} 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 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	Activator A	2 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Activator B	2 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Substrate	Powder × 1 vial	2-8℃ , 12 months
Reagent 5	Protein Precipitator	6 mL × 1 vial	2-8℃ , 12 months
Reagent 6	Chromogenic Agent A	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 7	Acid Reagent	10 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
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

▲Materials prepared by users

Instruments

Test tube, Vortex mixer, Incubator, Centrifuge, 100°C water bath, Microplate reader (660 nm)

Reagents:

Ultrapure 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.
3. To avoid external phosphorus contamination, be careful during the experiment.

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 reagent 4 with 5 mL double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 7 days.
3. Preparation of reagent 6 working solution:
   Dissolve a vial of reagent 6 with 5 mL double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 7 days with shading light.
4. Preparation of reagent 8 working solution:
   Dissolve a vial of reagent 8 with 5 mL double distilled water in 90-100°C water bath, cool to room temperature before use. The prepared solution can be stored at 2-8°C for 7 days.
5. Preparation of phosphorus assay reagent:
   Mix the double distilled water, reagent 6 working solution, reagent 7 and reagent 8 working solution at the ratio of 2:1:1:1 fully. 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 solution can be stored at 2-8°C for 5 days with shading light.
6. Preparation of 0.5 mmol/L standard:
   Dilute reagent 9 with double distilled water at a ratio of 1:19. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 7 days.

▲ Sample preparation

Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use. 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 (1.18-286.43 U/kg 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% Mouse liver tissue homogenate	1
10% Rat heart tissue homogenate	5-8
10% Rat lung tissue homogenate	5-8
10% Rat kidney tissue homogenate	5-8
10% Rat brain tissue homogenate	2-3

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	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
B	B	B	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'
C	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
D	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
E	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
F	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
G	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
H	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'

Note: A, blank wells; B, standard wells; S1-S46, control wells; S1'-S46', sample wells

▲Detailed operating steps

The measurement of samples

1. Incubation reaction

(1) Non-enzyme tube: Take 170 μL of reagent 1 to the 1.5 mL EP tube.

Enzyme tube: Take 170 μL of reagent 1 to the 1.5 mL EP tube.

(2) Add 40 μL of regent 2 to the non-enzyme tube, and add 40 μL of regent 3 to the enzyme tube.

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

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

(5) Incubate the each tube at 37℃ for 20 min.

(6) Add 50 μL of reagent 5 to the non-enzyme tube and mix fully. Then add 200 μL of sample to the non-enzyme tube. Add 50 μL of reagent 5 to the enzyme tube.

(7) Mix fully and centrifuge at 2000 g for 10 min, then take the supernatant for detection.

2. Chromogenic reaction

(1) Blank well: Take 20 μL of double distilled water to the corresponding wells.

Standard well: Take 20 μL of 0.5 mmol/L standard to the corresponding wells.

Control well: Take 20 μL of supernatant from non-enzyme tube to the corresponding wells.

Sample well: Take 20 μL of the supernatant from enzyme tube to the corresponding wells.

(2) Add 200 μL of phosphorus assay reagent to each well.

(3) Mix fully with microplate reader and incubate at 37℃ for 30 min. Measure the OD value of each well at 660 nm with microplate reader.

▲Summary operation table

1. Incubation reaction

	Non-enzyme tube	Enzyme tube
Regent 1 (μL)	170	170
Regent 2 (μL)	40	
Regent 3 (μL)		40
Regent 4 working solution (μL)	40	40
Sample (μL)		200
Mix fully and incubate at 37℃ for 20 min.		
Regent 5 (μL)	50	50
Sample (μL)	200	
Mix fully and centrifuge for 10 min, then take the supernatant for detection.		

2. Chromogenic reaction

	Blank well	Standard well	Control well	Sample well
Double distilled water (μL)	20			
0.5 mmol/L standard (μL)		20		
Supernatant of non-enzyme tube (μL)			20	
Supernatant of enzyme tube (μL)				20
Phosphorus assay reagent (μL)	200	200	200	200
Mix fully and incubate at 37℃ for 30 min with shading light. Measure the OD value at 660 nm.				

▲Calculation

1. Tissue sample(Calculated by tissue protein):

Definition: The amount of Ca^{2+} -ATPase in 1 g tissue protein per 1 hour that decompose the ATP to produce 1 mmol inorganic phosphorus at 37°C is defined as 1 unit.

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

2. Tissue sample(Calculated by tissue wet weight):

Definition: The amount of Ca^{2+} -ATPase in 1 kg wet weight per 1 hour that decompose the ATP to produce 1 mmol inorganic phosphorus at 37°C is defined as 1 unit.

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

Note:

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

A_1: $OD_{Standard} - OD_{Control}$

C: The concentration of standard, 0.5 mmol/L

T: The time of incubation reaction, 20 min=1/3 h

V_1: The total volume of incubation reaction, 0.5 mL

V_2: The volume of sample, 0.2 mL

V_3: The volume of normal saline homogenate, mL

m: The weight of tissue, g

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

For 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation table.

The results are as follows:

the average OD value of the control is 0.199, the average OD value of the sample is 0.215, the average OD value of the standard is 0.296, the average OD value of the blank is 0.051, the concentration of protein in sample is 8.96 gprot/L, and the calculation result is:

Ca^{2+} -ATP activity (U/gprot) = (0.215 - 0.199) ÷ (0.296 - 0.051) × 0.5 × 3 × (0.5 ÷ 0.2) ÷ 8.96 × 5= 0.13 U/gprot