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Ver.260801

Na⁺K⁺-ATPase Assay Kit

BC17002-01 (50T/24S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Na^+K^+ -ATPase is distributed widely in plants, animals, microorganisms and cells, which catalyzes the hydrolysis of ATP to ADP and inorganic phosphorus. The activity of ATPase can be detected by measuring the amount of inorganic phosphorus. Kinetic determination of lactate dehydrogenase according to the following reaction.

Kit components

Reagent	Volume	Storage
Reagent I	30mL	2-8°C
Reagent II	4mL	2-8°C
Reagent III	Powder x 2	-20°C
Dissolve thoroughly with 1mL of distilled water before use. The rest reagent can be kept at -20°C for one week.		
Reagent IV	2mL	2-8°C
Reagent V	3mL	2-8°C
Reagent VI	Powder	2-8°C
Dissolve thoroughly with 15mL of distilled water before use. The rest reagent can be kept at Na^+K^+ -20°C for one week.		
Reagent VII	Powder	2-8°C
Reagent VIII	15mL	RT
Standard solution (10μmol/mL)	1mL	2-8°C

Reagent Preparations

- **0.5μmol/mL standard phosphorus working solution:** Dilute the 10μmol/mL standard 20 times to 0.5μmol/mL standard with distilled water. For example: add 1.9mL of distilled water to 0.1mL of standard, mix thoroughly.
- **Phosphorus content determining reagent:** Prepare reagents for determining phosphorus content; make solution as the volume ratio of water: Reagent VI: Reagent VII: Reagent VIII 2:1:1:1, which should be light yellow. It lose efficacy if its colour change. Prepare the reagent when it will be used.

Note: It is better to use new beaker, glass rod and glass pipettes, or disposable plastic ware when making reagent to avoid phosphorus pollution.

Reagents and Equipment Required but Not Provide

Spectrophotometer, desk centrifuge, adjustable transferpettor, water bath, 1mL glass cuvette, mortar/homogenizer, ice and distilled water.

I. Sample Preparation

- **Bacteria or cells:** Collecting bacteria or cells into the centrifuge tube, centrifugation and discard the supernatant. Suggest that add 1mL of Reagent I to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cell (placed on ice, ultrasonic power 200W, ultrasonic 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000×g for 10minutes at 4°C to remove insoluble materials and take the supernatant on ice for test.
- **Tissue:** Add 1mL of Reagent I into 0.1g of tissue and fully grind on ice. Centrifuge at 8000xg for 10minutes at 4°C to remove insoluble materials and take the supernatant on ice for test.
- **Serum (plasma):** Detect directly.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 660nm, set the counter to zero with distilled water.

Note: Each test tube shall have one corresponding contrast tube.

2. Add the following reagents to EP tube

Reagent	Contrast tube (C)	Test tube (T)
Reagent I	130μL	90μL
Reagent II	80μL	80μL
Reagent III	40μL	40μL
Reagent IV	-	40μL
Sample	-	200μL
Mix thoroughly, then place the reaction solution in a 37°C (mammal) or 25°C (other species) water bath for 10 minutes.		
Reagent V	50μL	50μL
Sample	200μL	-
Mix thoroughly, centrifuge at 4000xg for 10 minutes at room temperature. Take the supernatant.		

3. Determination of phosphorus content, add the following reagents to 1.5mL EP tube:

Reagent(μL)	Blank tube (B)	Standard tube (S)	Contrast tube (C)	Test tube (T)
0.5μmol/mL Standard solution	-	100	-	-
Supernatant	-	-	100	100
Distilled water	100	-	-	-
Phosphorus content determining reagent	1000	1000	1000	1000

Mix thoroughly, then place the mix solution in the 40°C water bath for 10 minutes. Cooling to room temperature and detect the absorbance at 660nm as A(B), A(S), A(C), A(T). Each Test tube need to be provided with contrast tube, the standard curve and blank tube only need to be measured 1-2 times.

Calculation:

I. Serum (Plasma):

Unit definition: One unit of enzyme activity is defined as the amount of Na⁺K⁺-ATPase catalyzes the hydrolyzation of ATP to produce 1μmol of inorganic phosphorus in the reaction system per hour every milliliter serum (plasma).

$$\text{Na}^+\text{K}^+\text{-ATPase activity (U/mL)} = C_s \times [A(T)-A(C)] \div [A(S)-A(B)] \times V_{rv} \div s \div T \\ = 7.5 \times [A(T)-A(C)] \div [A(S)-A(B)]$$

II. Tissue, bacteria or cells

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of Na⁺K⁺-ATPase catalyzes the hydrolyzation of ATP to produce 1μmol of inorganic phosphorus in the reaction system per hour every milligram protein.

$$\text{Na}^+\text{K}^+\text{-ATPase activity (U/mg prot)} = C_s \times [A(T)-A(C)] \div [A(S)-A(B)] \times V_{rv} \div (V_s \times C_{pr}) \div T \\ = 7.5 \times [A(T)-A(C)] \div [A(S)-A(B)] \div C_{pr}$$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of Na⁺K⁺-ATPase catalyzes the hydrolyzation of ATP to produce 1μmol of inorganic phosphorus in the reaction system per hour every gram tissue.

$$\text{Na}^+\text{K}^+\text{-ATPase activity (U/g mass)} = C_s \times [A(T)-A(C)] \div [A(S)-A(B)] \times V_{rv} \div (V_s \div V_1 \times W) \div T \\ = 7.5 \times [A(T)-A(C)] \div [A(S)-A(B)] \div W$$

3. Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of Na⁺K⁺-ATPase catalyzes the hydrolyzation of ATP to produce 1μmol of inorganic phosphorus in the reaction system per hour every 10000 cells or bacteria.

$$\text{Na}^+\text{K}^+\text{-ATPase activity (U/10}^4\text{ cell)} = C_s \times [A(T)-A(C)] \div [A(S)-A(B)] \times V_{rv} \div (V_s \div V_1 \times 500) \div T \\ = 0.015 \times [A(T)-A(C)] \div [A(S)-A(B)]$$

C_s : Concentrate of standard tube, 0.5μmol/mL

V_{rv}: Total reaction volume, 0.5mL

V_s : Sample volume, 0.2mL

C_{pr} : Sample protein concentration (mg/mL)

T : Reaction time (min), 1/6 hour

W : Sample weight, g

V₁ : Volume of reagent I, 1mL

500 : The amount of bacteria or cells, 5 millions