



ISO 13485:2016 ISO 9001:2015

Ver.251201

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.

Kit components

Reagent	Volume	Storage
Reagent I	30mL	2-8°C
Reagent II	4mL	2-8°C
Reagent III	Powder x 2	-20°C
Reagent IV	2mL	2-8°C
Reagent V	3mL	2-8°C
Reagent VI	Powder x 1	2-8°C
Reagent VII	Powder x 1	2-8°C
Reagent VIII	15mL	RT
Standard solution (10 $\mu\text{mol}/\text{mL}$)	1mL x 1.10 $\mu\text{mol}/\text{mL}$	2-8°C

Reagent preparation

- **Reagent III** :Dissolve thoroughly with 1mL of distilled water before use. The rest reagent can be kept at - 20°C for one week.
- **Reagent VI**: 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**: Dissolve thoroughly with 15mL of distilled water before use, can be kept at 2-8°C for 2 weeks.
- **0.5 $\mu\text{mol}/\text{mL}$ standard phosphorus working solution**: Dilute the 10 $\mu\text{mol}/\text{mL}$ standard 20 times to 0.5 $\mu\text{mol}/\text{mL}$ standard with distilled water. For example: add 1.9 mL of distilled water to 0.1 mL 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 Provided

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

I. Sample Preparation

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, centrifugation and discard the supernatant. Suggest that add 1 mL 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.

2. Tissue

Add 1 mL of Reagent I into 0.1 g 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.

3. Serum (plasma): Detect directly.

II. Determination procedure:

- Preheat spectrophotometer for 30 minutes, adjust the wavelength to 660 nm, set the counter to zero with distilled water.
- Add the following reagents to EP tube:

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

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

Reagent	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 1 μmol of inorganic phosphorus in the reaction system per hour every milliliter serum (plasma).

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

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 1 μmol of inorganic phosphorus in the reaction system per hour every milligram protein.

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

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 1 μmol of inorganic phosphorus in the reaction system per hour every gram tissue.

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

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 1 μmol of inorganic phosphorus in the reaction system per hour every 10000 cells or bacteria.

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

C_s: Concentrate of standard tube, 0.5 μmol/mL

V_{rv}: Total reaction volume, 0.5 mL

V_s: Sample volume, 0.2 mL

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

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

W: Sample weight, g

V₁: Volume of Reagent I, 1 mL

500: The amount of bacteria or cells, 5 millions