Na^+K^+-ATPase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: BC0060

Size:50T/24S

Components:

Reagent I: Liquid 30 mL×1. Storage at 2-8℃.

Reagent II: Liquid 4 mL×1. Storage at 2-8℃.

Reagent III: Powder×2. Storage at -20℃. Dissolve thoroughly with 1 mL of distilled water before use. The rest reagent can be kept at -20℃ for one week.

Reagent IV: Liquid 2 mL×1. Storage at 2-8℃.

Reagent V: Liquid 3mL×1. Storage at 2-8℃.

Reagent VI: Powder×1. Storage at 2-8℃. Dissolve thoroughly with 15 mL of distilled water before use, the unused reagent can be stored at 2-8℃ for 2 weeks.

Reagent VII: Powder×1. Storage at 2-8℃. Dissolve thoroughly with 15 mL of distilled water before use, can be kept at 2-8℃ for 2 weeks.

Reagent VIII: Liquid 15 mL×1. Storage at RT.

Standard solution: Liquid 1 mL×1. 10 μmol/mL standard phosphorus liquid, storage at 2-8℃.

0.5 μmol/mL standard phosphorus working solution: Dilute the 10 μmol/mL standard 20 times to 0.5 μmol/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 H_2O: 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.

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.

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 and tissue:

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℃ to remove insoluble materials and take the supernatant on ice for test.

Tissue: Add 1 mL of Reagent I into 0.1 g of tissue and fully grind on ice. Centrifuge at 8000 ×g for 10minutes at 4℃ to remove insoluble materials and take the supernatant on ice for test.

2. Serum (plasma): Detect directly.

II. Determination procedure:

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

2. Add the following reagents to EP tube:

Reagent (μL)	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 ×g for 10 minutes at room temperature. Take the supernatant.		

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

Reagent (μL)	Blank tube (B)	Standard tub(S)	Contrast tube (C)	Test tube(T)
0.5 μmol/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℃-water bath for 10 minutes. Cooling to room temperature and detect the absorbance at 660 nm as A(B), A(S), A(C), A(T). Each Test tube needs to be provided with a contrast tube, the standard curve and blank tube only need to be measured 1-2 times.

II. Calculation:

1. 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

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system per hour every milliliter serum (plasma).

Na^+K^+-ATPase activity (U/mL) =Cs×[A(T)-A(C)] ÷[A(S)-A(B)] ×Vrv÷s÷T
=7.5×[A(T)-A(C)] ÷[A(S)-A(B)]

2. 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.

Na^+K^+-ATPase activity (U/mg prot) =Cs×[A(T)-A(C)] ÷[A(S)-A(B)] ×Vrv÷(Vs×Cpr) ÷T
=7.5×[A(T)-A(C)] ÷[A(S)-A(B)] ÷Cpr

(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.

Na^+K^+-ATPase activity (U/g mass) =Cs×[A(T)-A(C)] ÷[A(S)-A(B)] ×Vrv÷(Vs÷V1×W) ÷T
=7.5×[A(T)-A(C)] ÷[A(S)-A(B)] ÷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 1 μmol of inorganic phosphorus in the reaction system per hour every 10000 cells or bacteria.

Na^+K^+-ATPase activity (U/10^4 cell) =Cs×[A(T)-A(C)] ÷[A(S)-A(B)] ×Vrv÷(Vs÷V1×500) ÷T
=0.015×[A(T)-A(C)] ÷[A(S)-A(B)]

Cs: Concentrate of standard tube, 0.5 μmol/mL;

Vrv: Total reaction volume,0.5 mL;

Vs: Sample volume, 0.2 mL;

Cpr: 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.

Note:

1. As each sample needs one tube as contrast tube, this kit can detect 24 Na^+K^+-ATPase samples in 50 tubes.
2. This method has the characteristics of trace, sensitive and rapid. The test tubes used for determination are phosphate-free strictly.

Experimental examples:

1. Take 0.1 g of mouse heart and add 1 mL Reagent I for ice bath homogenization. After centrifugation at 4℃, 8000g, for 10 min, the supernatant is put on ice, and then operate according to the determination steps. The results showed that A(T)= 1.216, A(C) = 0.842, A(S) = 0.398, and A(B) = 0.004.

   Na^+K^+ - ATPase activity (U/g mass) = 7.5 × [A(T)-A(C)] ÷[A(S)-A(B)] ÷W= 71.19 U/g mass.
2. Take 0.1 g of barnyard grass and add 1 mL Reagent I to homogenize in ice bath. After centrifugation at 4℃, 8000g, for 10 min, the supernatant is put on ice, and then operate according to the determination steps. The results showed that A(T)= 0.474, A(C) = 0.403, A(S) = 0.398, and A(B) = 0.004

   Na^+K^+ - ATPase activity (U/g mass) = 7.5×[A(T)-A(C)] ÷[A(S)-A(B)] ÷ W = 13.52 U/g mass.
3. 200 μL of mouse plasma is diluted twice and detected directly, enzyme activity: A(T)= 0.958, A(C) = 0.906, A(S) =0.398, A(B) = 0.004

   Na^+K^+- ATPase activity (U/mL) = 7.5 ×[A(T)-A(C)] ÷[A(S)-A(B)] = 1.98 U/mL.

Recent product citations

[1] Lei L, Zhang F, Huang J, Yang X, Zhou X, Yan H, Chen C, Zheng S, Si L, Jose PA, Zeng C, Yang J. Selenium deficiency causes hypertension by increasing renal AT1 receptor expression via GPx1/H2O2/NF-κB pathway. Free Radic Biol Med. 2023 May 1; 200:59-72. doi: 10.1016/j.freeradbiomed. 2023.02.021. Epub 2023 Mar 2. PMID: 36868433; PMCID: PMC10164092.

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[4] Zeng M, Zhou H, He Y, Wang Z, Shao C, Yin J, Du H, Yang J, Wan H. Danhong injection alleviates cerebral ischemia/reperfusion injury by improving intracellular energy metabolism coupling in the ischemic penumbra. Biomed Pharmacother. 2021 Aug; 140:111771. doi: 10.1016/j.biopha.2021.111771. Epub 2021 May 28. PMID: 34058441.

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References:

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[2] Cornelius F. Modulation of Na, K-ATPase and Na-ATPase activity by phospholipids and cholesterol. I. Steady-state kinetics[J]. Biochemistry, 2001, 40(30): 8842-8851.

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[3] Gorini A, Canosi U, Devecchi E. et al. ATPases enzyme activities during ageing in different types of somatic and synaptic plasma membranes from rat frontal cerebral cortex[J]. Prog Neuropsychopharmacol Biol Psychiatry, 2002, 26(1): 81-90.

Related products:

BC0960/BC0965	$Ca^{++}Mg^{++}$ - ATPase Activity Assay Kit
BC0300/BC0305	ATP Content Assay Kit

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