Na^+K^+-ATPase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC0065

Size:100T/48S

Components:

Reagent I: Liquid 60 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. Prepare when the solution will be used. 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 5 mL of distilled water before use and the unused reagent can be stored at 2-8℃ for 2 weeks.

Reagent VII: Powder×1. Storage at 2-8℃. Dissolve thoroughly with 5 mL of distilled water before use and the unused reagent can be stored at 2-8℃ for 2 weeks.

Reagent VIII: Liquid 5 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/ microplate reader, micro glass cuvette/96 well flat-bottom plate, water bath, desk centrifuge, adjustable transferpettor, mortar, 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 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/ microplate reader for 30 minutes, adjust the wavelength to 660 nm, the spectrophotometer needs to be zeroed with distilled water.

2. Add the following reagents to EP tube:

Reagent (μL)	Contrast tube (C)	Test tube (T)
Reagent I	65	45
Reagent II	40	40
Reagent III	20	20
Reagent IV	-	20
Sample	-	100
Mix thoroughly, then place the reaction solution in a 37℃ (mammal) or 25℃ (other species) water bath for 10 minutes.		
Reagent V	25	25
Sample	100	-
Mix thoroughly, centrifuge at 4000 ×g for 10 minutes at room temperature. Take the supernatant.		

3. Determination of phosphorus content:

Reagent (μL)	Blank tube(B)	Standard tube(S)	Contrast tube(C)	Test tube(T)
0.5 μmol/ml standard solution	-	20	-	-
Supernatant	-	-	20	20
Distilled water	20	-	-	-
Phosphorus content determining reagent	200	200	200	200

Mix thoroughly, then place the mix solution in a 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 control 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

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) =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,0.25 mL;

Vs: Sample volume, 0.1 mL;

Cpr: Sample protein concentration (mg/mL);

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

W: Sample weight, g;

Vl: 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 48 Na^+K^+ -ATPase samples in 100 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 was put on ice, and then the 96 well plate is used to operate according to the determination steps. The results showed that A(T)= 0.741, A(C)= 0.509, A(S)= 0.280, and A(B)= 0.043.
   Na^+K^+ - ATPase activity (U/g mass) = 7.5 ×[A(T)-A(C)] ÷[A(S)-A(B)] ÷W= 73.42 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 was put on ice, and then the 96 well plate was used to operate according to the determination steps. The results showed that A(T)= 0.275, A(C)= 0.239, A(S)= 0.280, and A(B)= 0.043.
   Na^+K^+ - ATPase activity (U/g mass) = 7.5×[A(T)-A(C)] ÷[A(S)-A(B)]÷ W = 11.39 U/g mass.
3. 100 μL of mouse plasma is taken for detection, and 96 well plate is used to measure the enzyme activity: A(T)=1.114, A(C)= 1.054, A(S)= 0.280, A(B)= 0.043.
   Na^+K^+ - ATPase activity (U/mL) = 7.5 ×[A(T)-A(C)] ÷[A(S)-A(B)]= 1.90 U/mL.

Recent product citations

[1] Qiao Q, Liu X, Cui K, Li X, Tian T, Yu Y, Niu B, Kong L, Yang C, Zhang Z. Hybrid Biomimetic Nanovesicles to Drive High Lung Biodistribution and Prevent Cytokine Storm for ARDS Treatment. ACS Nano. 2022 Sep 27;16(9):15124-15140. doi: 10.1021/acsnano.2c06357. Epub 2022 Aug 29.

[2] Zhang W, Hu JJ, Liu R, Dai J, Yuan L, Liu Y, Chen B, Gong M, Xia F, Lou X. A Peptide-Conjugated Probe with Cleavage-Induced Morphological Change for Treatment on Tumor Cell Membrane. Adv Sci (Weinh). 2023 Apr;10(11): e2207228. doi: 10.1002/advs.202207228. Epub 2023 Feb 15. PMID: 36793151; PMCID: PMC10104630.

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[1] Luo L G, MacLean D B. Effects of thyroid hormone on food intake, hypothalamic Na/K ATPase activity and ATP content[J]. Brain research, 2003, 973(2): 233-239.

[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.

[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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