PRODUCT INFORMATION & MANUAL

Na+/K+ ATPase Activity Assay Kit (Colorimetric)

NBP3-25865

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

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（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Na+K+-ATPase Activity Assay Kit (Tissue And Cells)

Catalog No: NBP3-25865
Method: Colorimetric method
Specification: 100Assays **(**Can detect 48 samples without duplication)
Measuring instrument: Spectrophotometer

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used for detection of Na^+K^+-ATPase activity in animal tissue and culture cells samples.

Detection significance

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. The enzyme activity of ATPase will have a series of changes when the body in the hypoxic or diseases condition, and is also associated with some genetic diseases.

Detection principle

ATPase can decompose ATP and produce ADP and inorganic phosphorus. ATPase activity can be calculated indirectly by measuring the content of inorganic phosphorus.

Kit components

	Component	Specifications	Storage
Reagent 1	Liquid	13 mL ×2 vials	2-8℃, 12 months
Reagent 2	Liquid	4 mL ×2 vials	2-8℃, 12 months
Reagent 3	Powder	Powder ×8 vials	-20℃, 12 months
Preparation of reagent 3: dissolve 1 vial of Reagent 3 powder with 1 mL of double distilled water before use. Unused solution can be stored at -20℃ for a week.			
Reagent 4	Liquid	5 mL ×2 vials	2-8℃, 12 months
Reagent 5(A)	Solution A	7 mL ×8 vials	2-8℃, 12 months
Reagent 5(B)	Solution B	6 mL ×8 vials	2-8℃, 12 months, shading light
Note: There might be gelatinous substance in Solution B after long time storage in winter. It can't be dissolved at 37℃, it could be put in 60℃ water-bath for 10 min and would be dissolved totally. Solution A and Solution B should be protected from the contamination of phosphorus**.**			
Reagent 6	Liquid	50 mL ×2 vials	RT, 12 months
Reagent 7	10mmol/L Phosphorus Standard Stock Solution	5 mL ×1 vial	2-8℃, 12 months
Reagent 8	Stock Solution	0.1 mL ×4 vials	2-8℃, 12 months
	Diluent	0.9 mL ×4 vials	2-8℃, 12 months
Preparation of reagent 8: dilute a vial of stock solution with a vial of diluent. Unused solution can be stored at 2-8℃.			
Preparation of 0.1 μmol/mL standard application solution: dilute **reagent 7** with double distilled water for 100 times before use.			
Preparation of 0.02 μmol/mL Phosphorus standard solution: dilute **0.1 μmol/mL standard application solution** with double distilled water for 5 times before use.			

Preparation of substrate solution: mix reagent 1, reagent 2 and reagent 3 at a rate of 260:80:80. Prepare the fresh solution before use.
Preparation of chromogenic agent: mix reagent 5(A) and pre-warmed reagent 5(B) at a rate of 7:6 fully before 0.5 hour when use. The prepared chromogenic agent can be stored at 2-8℃ for at least 5 days. Please pay attention to avoid the contamination of phosphorus**.**

Experimental instruments

Test tube, Micropipettor, Vortex mixer, 37℃ water bath/gas bath, Spectrophotometer (636 nm)

Sample preparation

1. **Tissue sample**

   Weigh the tissue accurately, add 9 times of normal saline at the ratio of Weight (g): Volume (mL) = 1:9. Make the mechanical homogenization in ice water bath to prepare 10% homogenate. Centrifuge at 2500 rpm for 10 min and take the supernatant (10% homogenate). Then dilute the 10% homogenate with normal saline for 10 times to prepare 1% homogenate for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M is recommended). If the result of pre-experiment is too high, dilute the 1% tissue homogenate to different concentrations for pre-experiment and determine the sample concentration according to the result.

2. **Cells sample**

   Collect and centrifuge the culture cells, discard the supernatant and keep the cell sediment. Add 0.2~0.3 mL of normal saline to prepare 10^7/mL cell suspension, then broken the cells by homogenizer, ultrasonic crusher or freezing/thawing cycles. The prepared cell suspension does not need to be centrifuged. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M is recommended).Then dilute the cell homogenate to different concentrations for pre-experiment and determine the sample concentration according to the result.

[Note] 1. The sample must be mix fully when test the sample.

2. Do not use phosphate buffer or phosphorus-containing reagents as homogenization media or dilute samples.

3. The absolute OD value (the OD value of sample- the OD value of control) in pre-experiment should be control about 0.2.

4. The enzyme activity will be affect when treat the cells by freezing/thawing cycles.

Operation steps

1. Enzymatic reaction

	Control tube	Na^+K^+-ATPase sample tube
Double distilled water (mL)	0.16	0.12
Sample (mL)		0.1
Reagent 8 (mL)		0.04
Substrate (mL)	0.42	0.42
Mix fully, react for exactly 10 min at 37℃		
Reagent 4 (mL)	0.1	0.1
Sample (mL)	0.1	
Mix fully, centrifuge for 10 min at 3500 rpm and take the supernatant to determine phosphorus.		

2. Determine phosphorus

	Blank tube	Standard tube	Control tube	Na^+K^+-ATPase sample tube
Double distilled water (mL)	0.3			
0.02 μmol/mL phosphorus standard solution (mL)		0.3		
Supernatant (mL)			0.3	0.3
Chromogenic agent (mL)	1.0	1.0	1.0	1.0
Mix fully and stand for 2 min at room temperature.				
Reagent 6 (mL)	1.0	1.0	1.0	1.0
Mix fully and stand for 5 min at room temperature. Set the spectrophotometer to zero with double distilled water and Measure the OD values of each tube at 636 nm wavelength with 1 cm optical path cuvette.				

Note: cuvettes should be washed with tap water for 10 times, then washed with double distilled water for 4~5 times, avoid contaminated with phosphorus.

Calculation of results

1. **Definition:** The amount of ATPase in 1 mg of tissue protein that decompose ATP and produce 1 μmol inorganic phosphorus per hour is defined as 1 unit (μmol Pi/ mgprot /hour).
2. Na^+K^+-ATPase activity (*U/mgprot*)

   $$= \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times \text{Concentration of standard } (0.02\ \mu mol/mL)$$

   $$\times 6 \times 7.8 \div \text{Protein concentration of tested sampel } (mgprot/mL)$$

   [Note]

   6: The actual operation time is 10 min, while the reaction time in the definition is 1 h, so the result should be multiplied with 6.

   7.8: The dilution factor of enzymatic reaction.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. Avoid phosphorus pollution is the key for assay, it is recommended to use disposable test tubes.