CREATIVE BIOMART®
Assay Kit

Na+/K+ ATPase Assay Kit

Product Information

Cat.No. Kit-1031-2

Product Overview

Na+ /K+ ATPase is widely distributed in plants, animals, microbes and cells, can catalyze the hydrolysis of ATP, ADP and inorganic phosphate. Na+/K+ ATPase catalyze the decomposition of ATP into ADP and free phosphate ion. These enzymes play key roles in transport, signal transduction, protein biosynthesis and cell differentiation. At the end of the reaction period, the dye reagent forms a color with released phosphate ion, which is measured on a plate reader 660 nm.

Applications

Detection and Quantification of Na+ /K+ ATPase activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

Kit Components

96-Well Microplate, 1 plate
Assay Buffer, 30 ml x 4, 4 °C
Substrate, Powder x 1, -20 °C
Activator, Powder x 1, 4 °C
Inhibitor, Powder x 1, 4 °C
Dye Reagent ,I Powder x 1, 4 °C
Dye Reagent II, Powder x 1, 4 °C
Dye Reagent III, 20 ml x 1, 4 °C
Stop Solution, 4 ml x 1, RT
Standard (5 μmol/ml), 1 ml x 1, 4 °C
Plate Adhesive Strips, 3 Strips

Materials Required but Not Supplied

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips

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5. Mortar
6. Ice
7. Centrifuge

Preparation

REAGENT PREPARATION
Substrate: add 17 ml distilled water to dissolve before use, store at 4 °C.
Activator: add 1 ml distilled water to dissolve before use, store at 4 °C. (If it is difficult to dissolve, please heat up.)
Inhibitor: add 1 ml distilled water to dissolve before use, store at 4 °C. (If it is difficult to dissolve, please heat up.)
Dye Reagent: add 10 ml Dye Reagent III into Dye Reagent I and 2 ml Dye Reagent III into Dye Reagent II respectively to dissolve. Transfer all Dye Reagent II into Dye Reagent III, mix, then transfer all Dye Reagent I into Dye Reagent III (Must follow this step). The mixed Dye Reagent may store at 4 °C for 2-3 days.
*Note: It should be yellow. If colorless, the solution is failure. If blue, the solution is polluted. This solution should be prepared before use. It is best to use disposable plastic containers to prepare the solution in order to prevent phosphorus pollution.

SAMPLE PREPARATION
1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
3. For red blood cell samples Add heparin into the blood, centrifuged at 2000g 4 °C for 5 minutes. Discard the plasma and white blood cells. Wash the red blood cells with PBS for 3 times, discard the supernatant after centrifugation each time. Add 0.9 ml Assay buffer into 0.1 ml red blood cells, mix well, and wait for 15 minutes at room temperature.

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Assay Protocol

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Substrate	170 μl	170 μl	--	--
Sample	20 μl	20 μl	--	--
Inhibitor	--	10 μl		
Activator	10 μl	--		

Mix, put it in the oven, 37 °C for 30 minutes.

Stop Solution	40 μl	40 μl	--	--

Mix, centrifuged at 10000g, room temperature for 5 minutes. Add following reagents into the microplate:

Standard	--	--	20 μl	--
Distilled water	--	--	--	20 μl
Supernatant	20 μl	20 μl	--	--
Dye Reagent	180 μl	180 μl	180 μl	180 μl

Mix, room temperature for 30 minutes, record absorbance measured at 660nm.

Note:

1) It is best to use disposable plastic tube to avoid phosphorus pollution.
2) Perform 2-fold serial dilutions of the top standards to make the standard curve.
3) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

Analysis

Unit Definition: One unit of Na+/K+ ATPase activity is defined as the enzyme generates 1 μmol of PO4 3- per hour.

1. According to the protein concentration of sample

Na+/K+ ATPase (U/mg) = (CStandard × VTotal) × (ODSample - ODControl) / (ODStandard -

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ODBlank) / (VSample × CProtein) / T = 120 × (ODSample - ODControl) / (ODStandard - ODBlank) / CProtein

2. According to the weight of sample
Na+/K+ ATPase (U/g) = (CStandard × VTotal) × (ODSample - ODControl) / (ODStandard - ODBlank) / (W × VSample / VAssay) / T = 120 × (ODSample - ODControl) / (ODStandard - ODBlank) / W

3. According to the concentration of cell or bacteria
Na+/K+ ATPase (U/10^4) = (CStandard × VTotal) × (ODSample - ODControl) / (ODStandard - ODBlank) / (N × VSample / VAssay) / T = 120 × (ODSample - ODControl) / (ODStandard - ODBlank) / N
CProtein: the protein concentration, mg/ml;
W: the weight of sample, g;
CStandard: the concentration of Standard, 5 μmol/ml;
VTotal: the total volume of the enzymatic reaction, 0.24 ml;
VSample: the volume of sample, 0.02 ml;
VAssay: the volume of Assay buffer, 1 ml;
T: the reaction time, 0.5 h.

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