Na+ / K+ ATPase Assay Kit

Catalog No.: abx097985

Size: 96 tests

Detection Range: 0.42 µmol Pi/ml/hour – 4.99 µmol Pi/ml/hour

Sensitivity: 0.11 µmol Pi/ml/hour

Storage: Store all components at 4°C. Store Reagent A and Reagent B in the dark.

Application: For detection and quantification of Na+ / K+ ATPase activity in serum, plasma, whole blood, and tissue homogenates.

Introduction

Na+ / K+ ATPase, also known as the sodium-potassium pump, is an essential enzyme found on the membrane of all animal cells. Using energy provided by the hydrolysis of ATP to ADP, it drives the movement of sodium and potassium ions against their respective concentration gradients, establishing and maintaining an electrochemical gradient across the cell membrane. This gradient is essential to the functioning of numerous other enzymes, controlling osmotic pressure, facilitating the transduction of various cell signaling pathways, and the transmission of nerve signals.

Abbexa's Na+ / K+ ATPase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Na+ / K+ ATPase activity. As part of its enzymatic function, Na+ / K+ ATPase catalyzes the production of ADP and free phosphate ions from ATP. Phosphorous concentration can be tested, producing a red compound with an absorbance maximum at 660 nm. The intensity of the color is proportional to the Na+ / K+ ATPase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Diluent Buffer: 6 ml
3. Substrate: 1 vial
4. Reaction Buffer A: 5 ml
5. Reaction Buffer B: 1.5 ml
6. Precipitating Reagent: 2 × 1.5 ml
7. Acidic Reagent: 5 ml
8. Reagent A: 1 vial
9. Reagent B: 1 vial
10. Standard (10 µmol/ml Pi): 2 ml
11. Plate sealer: 2

Materials required but not provided

1. Microplate reader (660 nm)
2. Double distilled water
3. Normal (0.9% NaCl) saline
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Prepare samples according to conventional methods. If the samples contain significant amounts of suspension, centrifuge at 1,000 × g until clear, then test directly.
- **Whole blood:** Collect fresh blood into a tube containing EDTA or heparin and mix gently.
- **Tissue Homogenates:** Carefully weigh up to 1 g of tissue, and wash with cold normal (0.9%) saline. Add the tissue into normal saline in a ratio of 1:9 weight to volume (i.e. for every gram of tissue, add 9 ml normal saline). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes. Carefully remove the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
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Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal (0.9% NaCl) saline, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Rat plasma	1
10% Rat liver tissue homogenate	6 – 10
10% Rat spleen tissue homogenate	4 – 8
10% Rat heart tissue homogenate	4 – 8
10% Mouse brain tissue homogenate	4 – 8

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Dissolve the provided powder in 5 ml Double distilled water. The prepared solution can be stored at -20°C for up to 1 week.
- **Reagent A Working Solution:** Dissolve the provided powder in 5 ml Double distilled water. The prepared solution can be stored at 4°C in the dark for up to 1 week.
- **Reagent B Working Solution:** Dissolve the provided powder in 5 ml Double distilled water. The prepared solution can be stored at 4°C in the dark for up to 1 week.
- **Working Chromogenic Reagent:** In a fresh tube, mix Double distilled water, Reagent A Working Solution, Reagent B Working Solution, and the Acidic Reagent in a ratio of 2:1:1:1. This solution should be prepared just before use, and used within 24 hours.
- **Standards:** Label 7 tubes with 3.0 µmol/ml, 2.5 µmol/ml, 2.0 µmol/ml, 1.5 µmol/ml, 1.0 µmol/ml, 0.5 µmol/ml, and 0.25 µmol/ml. Add 300 µl, 250 µl, 200 µl, 150 µl, 100 µl, 50 µl, and 25 µl of Standard (10 µmol/ml Pi) to the 3.0 µmol/ml, 2.5 µmol/ml, 2.0 µmol/ml, 1.5 µmol/ml, 1.0 µmol/ml, 0.5 µmol/ml, and 0.25 µmol/ml tubes respectively, followed by 700 µl, 750 µl, 800 µl, 850 µl, 900 µl, 950 µl, and 975 µl of normal (0.9% NaCl) saline, to prepare the Standard Dilutions with concentrations 3.0 µmol/ml, 2.5 µmol/ml, 2.0 µmol/ml, 1.5 µmol/ml, 1.0 µmol/ml, 0.5 µmol/ml, and 0.25 µmol/ml. These volumes are summarized in the following table:

Standard Dilution (µmol/ml)	**3.0**	**2.5**	**2.0**	**1.5**	**1.0**	**0.5**	**0.25**
10 µmol/ml Pi Standard (µl)	300	250	200	150	100	50	25
Normal (0.9% NaCl) Saline (µl)	700	750	800	850	900	950	975

For the blank, or 0 µmol/ml standard, use pure normal (0.9% NaCl) saline. The volume of each standard will be 1000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- All glassware, disposable tubes, and pipette tips used to prepare these reagents must be completely free of phosphorous.

B. Assay Procedure

1. Mark microcentrifuge tubes for each sample and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 65 µl of Diluent Buffer to each control tube.
3. Add 45 µl of Diluent Buffer to each sample tube.
4. Add 40 µl of Reaction Buffer A to all tubes.
5. Add 20 µl of Substrate Working Solution to all tubes.
6. Add 20 µl of Reaction Buffer B to each sample tube, followed by 100 µl of the corresponding sample.
7. Mix fully for at least 3 seconds, and then incubate all tubes at 37°C for 10 minutes.
8. Add 25 µl of Precipitating Reagent to all tubes.
9. Add 100 µl of sample to each corresponding control tube.
10. Mix fully for at least 3 seconds, and then centrifuge all tubes at 8000 × g for 10 minutes. Carefully take the supernatant from each tube for analysis on the well plate.

11. Mark positions on the 96-well microplate for each sample, control, and standard.
12. Add 20 µl of each sample supernatant, control supernatant, and standard dilution to their corresponding wells.
13. Add 200 µl of Working Chromogenic Reagent to all wells.
14. Mix fully for 10 seconds by gently shaking the plate, then incubate at 37°C for 15 minutes.
15. Measure the OD of each well with a microplate reader at 660 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Na+ / K+ ATPase in each sample well can be derived with the formula:

1. Liquid samples (serum, plasma, and whole blood):

One unit of Na+ / K+ ATPase activity is defined as the amount required for 1 ml of serum or plasma to produce 1 µmol of inorganic phosphorous per hour.

$$\text{Na+ / K+ ATPase (µmol Pi/ml/hour)} = f \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Total}}}{\text{a} \times \text{T} \times \text{V}_{\text{Sample}}}$$

2. Tissue samples:

One unit of Na+ / K+ ATPase activity is defined as the amount required for 1 mg of tissue protein to produce 1 mg of reducing sugar per minute.

This calculation requires a measurement of total protein in the sample. This value must be determined separately from this assay.

$$\text{Na+ / K+ ATPase (µmol Pi/mg protein/hour)} = f \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Total}}}{\text{a} \times \text{T} \times \text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}}$$

where:

$\text{OD}_{\text{Sample}}$	OD value of sample
$\text{OD}_{\text{Control}}$	OD value of control
V_{Total}	Total volume of the reaction (0.25 ml)
V_{Sample}	Volume of sample added to the microplate (0.1 ml)
$\text{C}_{\text{Protein}}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = ax + b$)
b	Y-intercept of the standard curve ($y = ax + b$)
T	Time of the enzymatic reaction (10 minutes = 1/6 hour)
f	The dilution factor of sample