CheKine™ Micro Na^+/K^+-ATPase Activity Assay Kit

Cat #: KTB1800 Size: 48 T/96 T

	Micro Na^+/K^+-ATPase Activity Assay Kit		
REF	**Cat #:** KTB1800	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

The Na^+/K^+-ATPase is an enzyme widely distributed in biomembrane system of the organism. It performs several functions in cell physiology and it can catalyze ATP hydrolysis to produce ADP and inorganic phosphorus. CheKine™ Micro Na^+/K^+-ATPase Activity Assay Kit provides a simple method for detecting Na^+/K^+-ATPase activity in a variety of biological samples such as Serum, Plasma, Animal and Plant Tissues and Cells, Cell Supernatant, Bacteria. In the assay, Na^+/K^+-ATPase catalyzes ATP hydrolysis to produce ADP and inorganic Phosphorus. The content of inorganic phosphorus can reflect the activity of ATPase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	5 mL	10 mL	4℃
Reagent Ⅰ	1	1	-20℃, protected from light
Reagent Ⅱ	1 mL	2 mL	4℃
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	1	1	4℃, protected from light
Reagent Ⅴ	1	1	4℃, protected from light
Reagent Ⅵ	25 mL	25 mL	4℃
Reagent Ⅶ	5 mL	10 mL	4℃

Materials Required but Not Supplied

• Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm

- Incubator
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent Ⅰ: Add 6 mL deionized water before use. Store at -20℃, protected from light.
Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
ReagentⅢ: Add 3 mL deionized water before use. Store at 4℃, protected from light.
ReagentⅣ: Add 25 mL deionized water before use. Store at 4℃, protected from light.
Reagent Ⅴ: Add 25 mL deionized water before use. Store at 4℃, protected from light.
ReagentⅥ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
ReagentⅦ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
0.5 μmol/mL Phosphorus Standard Solution: Dilute ReagentⅦ by 20 times. The specific operation method is to add 0.1 mL ReagentⅦ to 1.9 mL deionized water, and mix well.
Phosphorus Fixing Reagent: According to the ratio of deionized water: ReagentⅣ : Reagent Ⅴ : ReagentⅥ=2: 1: 1: 1, the prepared Phosphorus Fixing Reagent should be light yellow. If the color of the prepared reagent is colorless, the reagent is invalid, and if blue, it is Phosphorus contamination. Always prepare fresh Phosphorus Fixing Reagent for every use.
Notes: The ReagentⅣ and Reagent Ⅴ can be stored at 4℃ for one week after dissolution. It is better to use new beakers, glass rods and glass pipettes, or use disposable plastic containers to prepare reagents to avoid phosphorus contamination.

Sample preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. Processed samples must be assayed immediately.
1. Serum, Plasma or other Liquid samples: Directly test.
2. Animal Tissue samples: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay. Keep it on ice to be tested.
3. Cell or Bacteria samples: Collect appropriate number of cells or bacteria for each assay, centrifuge at 12,000 g for 1 min at 4°C and discard the supernatant. Add 1 mL Extraction Buffer for every 5×10^6 cells or bacteria. Ultrasonic wave breaks cells or bacteria (ice bath, power 20% or 200 w, ultrasonic wave 3 s, interval 10 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay. Keep it on ice to be tested.
4. Plant Tissue samples: Wash plant with cold PBS to remove impurities as much as possible. Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay. Keep it on ice to be tested.
Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.
2. Enzyme catalysis (add the following reagents to the EP tube):

Reagent	Control Tube (μL)	Test Tube (μL)
Assay Buffer	65	45
Reagent Ⅰ	60	60
Reagent Ⅱ	0	20
Sample	0	100
Mix well, put into water bath at 37℃ (for mammals) or 25℃ (for other species) for 10 min		
Reagent Ⅲ	25	25
Sample	100	0
Mix well, centrifuge at 4,000 g for 10 min at room temperature, and take the supernatant		

3. Determination of Phosphorus (add the following reagents to the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Control Well (μL)	Test Well (μL)
0.5 μmol/mL Phosphorus Standard Solution	0	20	0	0
Supernatant	0	0	20	20
Deionized Water	20	0	0	0
Phosphorus Fixing Reagent	200	200	200	200

4. Mix well, keep at room temperature for 30 min and measure absorbance at 660 nm, record the optical density value of each well.

Note: Every sample needs to set a control well. Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test}-$A_{Control}$ is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of Na^+/K^+-ATPase active in serum, plasma or other liquid samples

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by Na^+/K^+-ATPase per milliliter of serum (plasma) per hour is defined as an enzyme activity unit.

Na^+/K^+-ATPase activity (U/mL)=$C_{Standard}\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\times V_{Total}\div V_{Sample}\div(T\div60)$

$=7.5\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})$

2. Calculation of Na^+/K^+-ATPase active in tissues, bacteria or cells

(1) Calculated by protein concentration

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by Na^+/K^+-ATPase per mg tissue protein per hour is defined as an enzyme activity unit.

Na^+/K^+-ATPase activity (U/mg)=$C_{Standard}\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\times V_{Total}\div(Cpr\times V_{Sample})\div(T\div60)$

$=7.5\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\div Cpr$

(2) Calculated by fresh weight of samples

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by Na^+/K^+-ATPase per gram of tissue per hour is defined as an enzyme activity unit.

Na^+/K^+-ATPase activity (U/g)=$C_{Standard}\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\times V_{Total}\div(V_{Sample}\div V_{SampleTotal}\times W)\div(T\div 60)$

$=7.5\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\div W$

(3) Calculated by bacteria or cells density

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by Na^+/K^+-ATPase per 10^4 bacteria or cells per hour is defined as an enzyme activity unit.

Na^+/K^+-ATPase activity (U/10^4)=$C_{Standard}\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times 500)\div(T\div 60)$

$=0.015\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})$

Where: $C_{Standard}$: Concentration of Phosphorus standard solution, 0.5 μmol/mL; V_{Total}: The total volume of enzymatic reactions, 0.25 mL; V_{Sample}: The volume of sample added to the reaction system, 0.1 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added to samples, 1 mL; T: Reaction time, 10 min; Cpr: Protein concentration of the sample, mg/mL.W: Fresh weight of sample, g. 500: Total number of bacteria or cells, 5×10^6.

Recommended Products

Catalog No.	Product Name
KTB1810	CheKine™ Micro Ca^{2+}/Mg^{2+}-ATPase Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.