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CheKine™ Micro ATPase Activity Assay Kit

Cat #: KTB1811 Size: 48 T/48 S 96 T/96 S

	Micro ATPase Activity Assay Kit		
REF	**Cat #:** KTB1811	LOT	**Lot #:** Refer to product label
	Detection range: 0.015625-1 μmol/mL		**Sensitivity:** 0.015625 μmol/mL
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

ATPase is a class of key enzymes widely present in various tissues and cells of organisms. By catalyzing the hydrolysis of ATP to ADP and inorganic phosphate (Pi), it provides direct energy for vital life activities such as transmembrane transport of substances, energy conversion, and signal transduction. The activity level of ATPase is closely related to the cellular energy metabolic state, and its activity changes are often used as important indicators for evaluating cell function impairment, tissue hypoxia, and certain genetic diseases.

The principle is based on the coupling of the ATPase-catalyzed enzymatic reaction and the inorganic phosphate colorimetric reaction. ATPase in the sample hydrolyzes the substrate ATP to generate ADP and inorganic phosphate (Pi). In the reaction system, the inorganic phosphate released by ATP hydrolysis reacts with the chromogenic reagent to form a blue-colored complex, which has a characteristic absorption peak at 660 nm. By measuring the absorbance at 660 nm, the ATPase activity level in the sample can be calculated based on the quantitative relationship in which the absorbance change is proportional to the ATPase activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	7.5 mL	15 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	5 mL	10 mL	4℃, protected from light
Reagent Ⅳ	25 mL	50 mL	4℃, protected from light
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	500 μL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Water bath, ice maker, freezing centrifuge, Sonication homogenizer (for cells or bacteria)
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent II: Prepared before use. Add 1.25 mL deionized water for 48 T and 2.5 mL deionized water for 96 T to fully dissolve. The prepared reagent can be stored at -20℃ for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent III: Ready to use as supplied; Equilibrate to room temperature before use. Store aliquots at 4℃, protected from light.

Reagent IV: Ready to use as supplied; Equilibrate to room temperature before use. Store aliquots at 4℃, protected from light.

Working Reagent V: Prepared before use. Add 7.5 mL deionized water for 48 T and 15 mL deionized water for 96 T to fully dissolve. The prepared reagent can be stored at -20℃ for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Standard: Ready to use as supplied; Equilibrate to room temperature before use. 10 μmol/mL standard solution; Store at 4℃, protected from light.

Standard preparation:

Using 10 μmol/mL standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Deionized water (μL)	Concentration (μmol/mL)
Std.1	100 μL of 10 μmol/mL Standard	900	1
Std.2	200 μL of Std.1 (1 μmol/mL)	200	0.5
Std.3	200 μL of Std.2 (0.5 μmol/mL)	200	0.25
Std.4	200 μL of Std.3 (0.25 μmol/mL)	200	0.125
Std.5	200 μL of Std.4 (0.125 μmol/mL)	200	0.0625
Std.6	200 μL of Std.5 (0.0625 μmol/mL)	200	0.03125
Std.7	200 μL of Std.6 (0.03125 μmol/mL)	200	0.015625
Blank	0	200	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: It is recommended to use fresh samples and extract them as soon as possible. If extraction cannot be performed on the same day, store intact cells or aliquoted tissue pieces at -80℃ and use within one month. When ready to perform the experiment, thaw the samples on ice after removing them from -80℃. However, please note that this may affect sample stability, and the experimental results may be lower than expected.

1. Animal or Plant Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the

supernatant after centrifugation **(Note: Ensure the removal of any residual liquid droplets from the tube mouth and wall)**; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Plasma, Serum or other Liquid samples: Test directly. If turbidity occurs, centrifuge before detection.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

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. Working Reagent: Prepare immediately before use. To prepare the working solution, mix 160 μL of Reagent Ⅳ with 40 μL of Working Reagent Ⅴ per well (volume ratio 4:1) to give a total of 200 μL. Mix thoroughly. Prepare fresh and use immediately. The prepared working solution should be mixed again before use.

3. Enzymatic reaction (add the following reagents respectively into each EP tube). **This step is not required for blank tube and standard tube.**

Reagent	Test Tube (μL)	Control Tube (μL)
Reagent Ⅰ	40	40
Reagent Ⅱ	10	10
Sample	50	0
Mix well, then cap tightly and incubate at 37°C for exactly 15 minutes		
Reagent Ⅲ	20	20
Sample	0	50

4. Mix well, Centrifuge at 7,000 g for 10 min at 4℃, collect the supernatant to new EP tubes for testing.

5. Detection System Preparation: add the following reagent into the 96-well plate or microglass cuvette carefully.

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Supernatant	0	0	40	40
Standards	0	40	0	0
Deionized Water	40	0	0	0
Working Reagent	200	200	200	200

6. Mix well, stand at room temperature (25 ℃)for 10 min. Then read the absorbance at 660 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. Blank Well and Standard Well only need to be measured 1 time.

Note: 1. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.If the measured ΔATest is less than 0.01, the sample volume may be appropriately increased. If the measured ΔA_{Test} exceeds the $\Delta A_{Standard}$ corresponding to the 1 μmol/mL standard, the sample can be further diluted with Extraction Buffer, or the sample amount used for extraction can be reduced and the extraction repeated.

2. If multiple samples are detected simultaneously, use a multi-channel pipette can increase the speed of sample addition.

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. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculation of ATPase activity

Bring the ΔA_{Test} of the sample into the equation to get the y value (μmol/mL).

(1) Calculated by fresh weight of samples

Unit definition: one enzyme activity unit defines as 1 μmol of inorganic phosphorus produced by per g tissue per minute in the reaction system.

ATPase activity (U/g)=$y \times V_{Enzymatic\ Reaction} \div (V_{Sample} \div V_{Sample\ total} \times W) \div T$=**$0.16 \times y \div W$**

(2) Calculated by protein concentration

Unit definition: one enzyme activity unit defines as 1 μmol of inorganic phosphorus produced by per mg tissue protein per minute in the reaction system.

ATPase activity (U/mg prot)=$y \times V_{Enzymatic\ Reaction} \div (V_{Sample} \times Cpr) \div T$=**$0.16 \times y \div Cpr$**

(3) Calculated by volume of liquid samples

Unit definition: one enzyme activity unit defines as 1 μmol of inorganic phosphorus produced by per liter of liquid per minute in the reaction system.

ATPase activity (U/L)=$y \times V_{Enzymatic\ Reaction} \div (V_{Sample} \times 10^{-3}) \div T$**$=160 \times y$**

(4) Calculated by number of cells or bacteria

Unit definition: one enzyme activity unit defines as 1 μmol of inorganic phosphorus produced by per 10^6 cells per minute in the reaction system..

ATPase activity (U/10^6)=$y \times V_{Enzymatic\ Reaction} \div (V_{Sample} \div V_{Sample\ total} \times N) \div T$**$=0.16 \times y \div N$**

Where: $V_{Enzymatic\ Reaction}$: total Enzymatic reaction volume, 0.12 mL; Cpr: Sample protein concentration, mg/mL; 10^{-3}: Unit conversion factor, 1 mL=10^{-3} L; V_{Sample}: sample volume added, 0.05 mL; T: reaction time, 15 min; $V_{Extraction}$: sample extract volume, 1 mL; W:sample weight, g; N: Number of bacteria or cells, use 10^6 as the unit (e.g., total number of cells: 5×10^6, N=5).

Precautions

1. For samples with low activity, such as cells or bacteria, the enzymatic reaction time can be extended to 30 min or 1 h to increase product accumulation and facilitate detection.

Typical Data

Typical standard curve

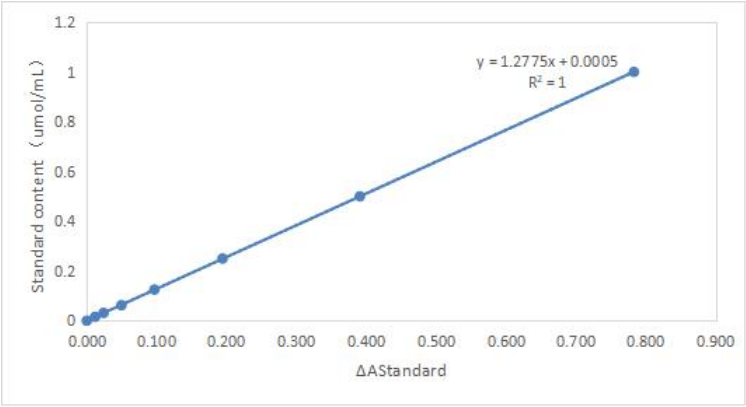

Figure 1. Standard Curve of ATPase Activity.

Example-1: 0.1 g of mouse liver was taken, diluted 5-fold with Extraction Buffer, and processed according to the assay procedure. Measurements were made using a 96-well plate, yielding: $\Delta A_{Test}=A_{Test}-A_{Control}=0.801–0.220=0.581$. Substituting this value into the standard curve gave y=0.743. Based on the sample weight, the ATPase activity was calculated as: ATPase activity (U/g)=0.16×y÷W=1.189 U/g, where W=0.1 g.

Recommended Products

Catalog No.	Product Name
KTB1810	CheKine™ Micro Ca^{2+}/Mg^{2+}-ATPase Activity Assay Kit
KTB1800	CheKine™ Micro Na^{+}/K^{+}-ATPase Activity Assay Kit
KTB1016	CheKine™ Micro ATP Content Assay Kit

Disclaimer

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