CheKine™ Micro ATP Content Assay Kit

Cat #: KTB1016 Size: 48 T/48 S 96 T/96 S

	Micro ATP Content Assay Kit		
REF	**Cat #:** KTB1016	LOT	**Lot #:** Refer to product label
	Detection range: 0.02-8 μmol/mL		**Sensitivity:** 0.01 μmol/mL
	Applicable samples: Animal Tissues, Plant Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

ATP exists widely in animals, plants, microorganisms and cultured cells. It is the biological energy currency, and energy charge is the main parameter to describe the state of cell energy metabolism. Determination of ATP content and calculation of energy charge can reflect the state of energy metabolism. CheKine™ Micro ATP Content Assay Kit can detect the ATP concentration from liquid samples, such as animal and plant tissues, cells, bacteria, and serum (plasma). The principle is that creatine kinase catalyse the reaction of creatine and ATP to creatine phosphate. Phosphomolybdic acid colorimetry can detect creatine phosphate content at 700 nm to reflect ATP content.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅱ	2.5 mL	5 mL	4℃
Reagent Ⅲ	18 μL	36 μL	-20℃, protected from light
Reagent Ⅳ	5 mL	10 mL	4℃, protected from light
Reagent Ⅴ	25 mL	50 mL	4℃
Standard	Powder×1 vial	Powder×1 vial	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 700 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, water bath
- Deionized water

• Homogenizer (for tissue samples)

Reagent Preparation

Reagent I: Before use, add 1.2 mL deionized water for 48 T, add 2.4 mL deionized water for 96 T, heat and boil until completely dissolved. The rest of the reagent is stored at -20℃ for 4 weeks after aliquoting to avoid repeated freezing and thawing.
Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Working Reagent III: Before use, add 0.6 mL deionized water for 48 T, add 1.2 mL deionized water for 96 T and mix well. The rest of the reagent is stored at -20℃, protected from light for 4 weeks after aliquoting to avoid repeated freezing and thawing.
Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent V: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Note: Reagent IV has a pungent odor, Reagent V is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.
Working Reagent: Prepared before use; according to the ratio of Reagent IV: Reagent V=1:5, and use freshly according to need.
Standard: Before use, add 10 mL deionized water to dissolve, and the concentration is 2 μmol/mL ATP standard solution. The rest of the reagent is stored at -20℃, protected from light for 4 weeks after aliquoting to avoid repeated freezing and thawing.
Note: The experiment should not have any phosphorus pollution, so it is recommended to use disposable plastic utensils.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.
1. Plant or animal tissue samples: Weigh 0.1 g tissues and add 1 mL deionized water. Homogenize on ice. Heat at 100℃ for 5 min. Centrifuge at 8,000 g for 15 min at 4℃. Use supernatant for assay.
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; add 1 mL deionized water to ultrasonically disrupt the cells or bacteria 1 min (power 20% or 200 W, ultrasonic 2 s, interval 1 s, repeat 20 times). Heat at 100℃ for 5 min. Centrifuge at 8,000 g for 15 min at 4℃. Use supernatant for assay.
3. Serum or plasma sample: Take 0.1 mL sample and add 1 mL deionized water, mix well. Heat at 100℃ for 5 min. Centrifuge at 8,000 g for 15 min at 4℃. Use supernatant for assay.
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 700 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each well :

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	10	10
Standard	10	10	0	0
Reagent I	0	20	20	0
Reagent II	10	10	10	10
Working Reagent III	0	10	10	0
Deionized Water	30	0	0	30

Mix well and incubate at 37℃ for 30 min				
Working Reagent	200	200	200	200
After incubation at 37℃ for 20 min, the absorption value was measured at 700 nm wavelength. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: (1) Only one Blank Well and Standard Well are usually made. (2) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. (3) The final reaction color of the system is blue or yellow-green, which is a normal phenomenon and does not affect the detection result of OD value.

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. Calculating the content of ATP

(1) Calculated by the volume of serum (plasma)

ATP (μmol/mL)=$[C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V1]\div(V3\times V1\div V2)$**=$20\times\Delta A_{Test}\div\Delta A_{Standard}$**

(2) Calculated by fresh weight of samples

ATP (μmol/g fresh)=$[C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V1]\div(W\times V1\div V2)$**=$2\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

(3) Calculated by sample protein concentration

ATP (μmol/mg prot)=$[C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V1]\div(V1\div Cpr)$**=$2\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

(4) Calculated by number of cells or bacteria

ATP (μmol/10^6)=$[C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V1]\div(5\times V1\div V2)$**=$0.4\times\Delta A_{Test}\div\Delta A_{Standard}$**

Where: $C_{Standard}$: the concentration of the standard, 2 μmol/mL; V1: add sample volume, 0.01 mL; V2: add deionized water volume to sample, 1 mL; V3: add serum (plasma) volume: 0.1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 5: Total number of cells or bacteria, 5×10^6.

Recommended Products

Catalog No.	Product Name
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit
KTB1330	CheKine™ Micro Blood Glycogen Assay Kit
KTB1340	CheKine™ Micro Glycogen Assay Kit
KTB1350	CheKine™ Micro Total Carbohydrate Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.