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CheKine™ ATP Citrate Lyase (ACL) Activity Assay Kit

Cat #: KTB1252 Size: 48 T/96 T

	Micro ATP Citrate Lyase (ACL) Activity Assay Kit		
REF	**Cat #:** KTB1252	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

ATP citrate lyase is a key cytosolic enzyme that catalyzes the production of acetyl-CoA from citric acid. Produced acetyl-CoA is the main raw material for the synthesis of fatty substances such as fatty acids and cholesterol, and can participate in the modification of related important proteins. It is a pivotal substance for energy substance metabolism in the body. CheKine™ Micro ATP Citrate Lyase (ACL) Activity Assay Kit can be used to detect biological samples such as animal and plant tissue, cells or bacteria, serum or plasma. In this kit, in the presence of ATP and coenzyme A, ACL can catalyze the cleavage of citric acid into acetyl coenzyme A, oxaloacetate, ADP, and phosphate. Malate dehydrogenase further catalyzes oxaloacetate and NADH to produce malate and NAD^+, leading light absorption decreases at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tubes
- Water bath, freezing centrifuge
- Deionized water
- Mortar or homogenizer (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℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃ protected from light.

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

Working Reagent: Prepared before use. Transfer Reagent II and Reagent III to Reagent I and fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize 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; 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. Serum, Plasma: Test directly.

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 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheat Working Reagent at 37℃ (mammals) or 25℃ (other species) for 5 min.
3. Operation table (The following operations are operated in the 96-well UV plate or microglass cuvette):

Reagent	Test Well (μL)
Sample	20
Working Reagent	180

4. Mix well, immediately record the absorption value A_1 at 20 s at 340 nm and A_2 after 320 s. Finally calculate $\Delta A=A_1-A_2$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.02, increase the sample quantity appropriately or prolong reaction time. If ΔA is greater than 0.4, 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.

Calculation of the ACL activity

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Unit definition: The consumption of 1 nmol NADH per min in mg protein is defined as a unit of enzyme activity.

ACL(U /mg prot) = [ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T=**643.08×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: The consumption of 1 nmol NADH per min in g tissue is defined as a unit of enzyme activity.

ACL (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ Sample}$)÷T=**643.08×ΔA÷W**

(3) Calculated by bacteria or cell of samples

Unit definition: The consumption of 1 nmol NADH per min in 10^4 bacteria or cells is defined as a unit of enzyme activity.

ACL (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(n×V_{Sample}÷$V_{Total\ Sample}$)÷T=**643.08×ΔA÷n**

(4) Calculated by volume of liquid samples

Unit definition: The consumption of 1 nmol NADH per min in mL liquid is defined as a unit of enzyme activity.

ACL(U /mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T=**643.08×ΔA**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol /cm; d: 0.5 cm; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: the volume of adding Extraction Buffer, 1 mL; T: reaction time, 5 min; W: sample weight, g; n: total number of bacteria or cells, calculated in units of ten thousand.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

The following data are for reference only, and the experimenter is required to test the samples according to their own experiments.

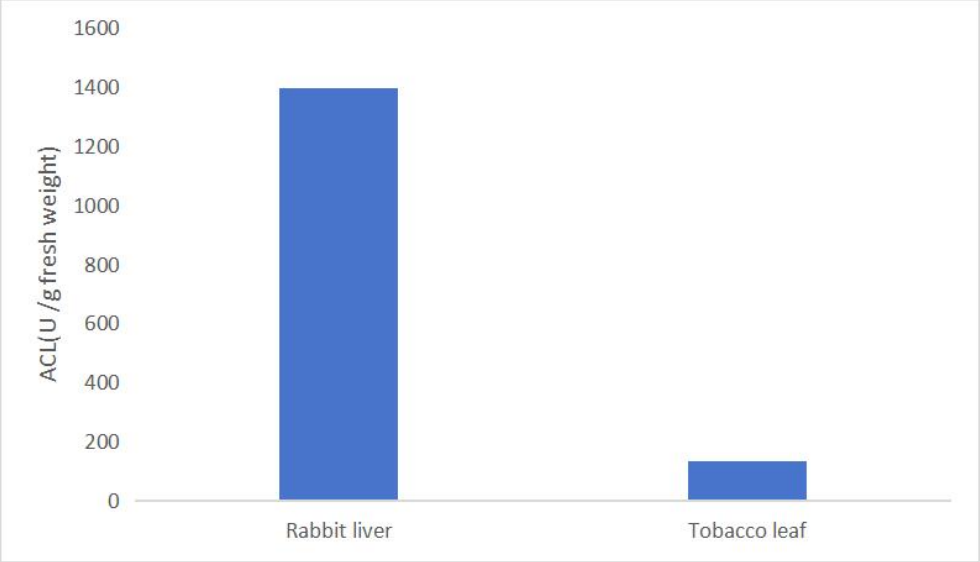

Figure 1. Determination ACL activity in rabbit liver and tobacco leaf by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1261	CheKine™ Micro Acetyl CoA Carboxylase (ACC) Activity Assay Kit
KTB2220	CheKine™ Micro Total Cholesterol (TC) Content Assay Kit
KTB3032	CheKine™ Acetaldehyde Dehydrogenase (ALDH) Activity Assay Kit

Disclaimer

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