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CheKine™ Micro Citric Acid (CA) Content Assay Kit

Cat #: KTB1024 Size: 48 T/96 T

	Micro Citric Acid (CA) Content Assay Kit		
REF	**Cat #:** KTB1024	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Bacteria, Mitochondria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

CA is a common organic acid in organism and an important food flavor substance. In addition, CA is the product of the first step of the tricarboxylic acid cycle. CheKine™ Micro Citric Acid (CA) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria, mitochondria, serum or plasma. In the kit, under acid condition, Cr^{6+} is reduced to Cr^{3+} by citric acid, which have a characteristic absorption peak at 545 nm. The content of citric acid in the sample can be calculated by measuring the increase of the absorption value at 545 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	120 mL	4℃, protected from light
Reagent Ⅱ	11 mL	22 mL	4℃
Reagent Ⅲ	0.25 mL	0.5 mL	-20℃, protected from light
Reagent Ⅳ	1	1	RT, protected from light
Reagent Ⅴ	1.06 mL	2.12 mL	4℃, protected from light
Standard	0.25 mL	0.25 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 545 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL eppendorf tube
- Water bath pot, cryogenic centrifuge machine
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

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

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use; It is a volatile reagent and should be sealed as soon as possible after use.Store at -20℃, protected from light.

Reagent IV: Prepared before use. 48 T add 1.25 mL Reagent Ⅰ, 96 T add 2.5 mL Reagent Ⅰ, fully dissolve, and store the inexhaustible reagents at 4℃ for 2 weeks, protected from light.

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

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

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 Reagent Ⅰ and homogenize on ice. Centrifuge at 11,000 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 bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ to ultrasonically disrupt the cells or bacteria 3 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s). Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Extraction of citric acid from Mitochondria: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice. Centrifuge at 600 g for 5 min at 4℃. Take the supernatant to another centrifugal tube, centrifuge at 11,000 g for 10 min at 4℃ and discard the supernatant(This supernatant can be used for the determination of CA in cytoplasm). Add 200 μL Reagent Ⅱ and 2 μL Reagent Ⅲ to the precipitation to fully suspend and dissolve. Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma, Serum or other Liquid samples: Take 0.1 mL liquid and add 0.9 mL Reagent Ⅰ, mix well. Centrifuge at 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 545 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat 30 min with Reagents Ⅰ at 30℃.
3. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	20
Standard	0	20	0
Deionized Water	20	0	0
Reagent Ⅰ	140	140	140
Reagent Ⅳ	20	20	20
Reagent Ⅴ	20	20	20

4. After fully mixing, leave it for 30 min at room temperature, measure the absorbance at 545 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Well and the Blank well only need to be tested 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If ΔA is greater than 0.5, the sample can be appropriately diluted with deionized water, 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 CA content

(1) Calculated by sample protein concentration

$CA(\mu mol/mg\ prot)=C_{Standar}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div(Cpr\times V_{Sample})=\mathbf{2\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by fresh weight of samples

$CA(\mu mol/g\ fresh\ weight)=C_{Standar}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ Sample}\div W=\mathbf{2\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

(3) Calculated by cells

$CA(\mu mol/10^4\ cell)=C_{Standar}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ Sample}\div n=\mathbf{2\times\Delta A_{Test}\div\Delta A_{Standard}\div n}$

(4) Calculated by volume of liquid samples

$CA(\mu mol/mL)=C_{Standar}\times\Delta A_{Test}\div\Delta A_{Standard}\times F=\mathbf{20\times\Delta A_{Test}\div\Delta A_{Standard}}$

$C_{Standard}$: Concentration of standard substance, 2 μmol/mL; V_{Sample}: Added sample volume, 0.02 mL; $V_{Total\ Sample}$: Total volume of supernatant; Cpr: Protein concentration of supernatant, mg/mL; W: Sample mass, g; n: The number of cells; F: Sample dilution times, (0.1 mL sample+0.9 mL Reagent Ⅰ)÷0.1 mL sample=10.

Precautions

1. Reagent Ⅴ is a carcinogen. During the experiment, gloves should be worn to avoid Reagent Ⅴ splashing on the skin.
2. If there are obvious small black particles after 30 min of reaction, it is a normal phenomenon, the sample should be diluted and then measured.
3. Citric acid extract can not be used for protein content determination, if you need to determine protein content, you need to take another tissue.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

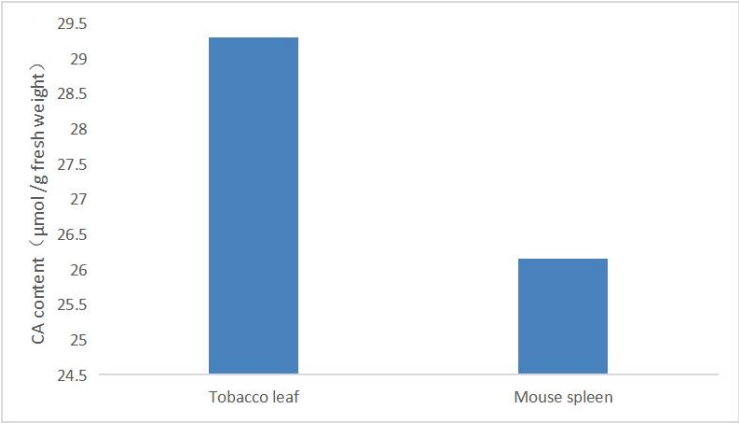

Figure 1. Determination of CA content in tobacoo leaf mouse spleen by this kit.

Recommended Products

Catalog No.	Product Name
KTB1230	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

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