

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Citric Acid (CA) Assay Kit

Catalog No.: BC00136

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Citric Acid (CA) Assay Kit
Detection Method	Colorimetric
Sample Type	Fluids, tissues, cells, mitochondrial proteins
Assay Type	Quantitative
Detection Instrument	Microplate reader (412 nm)

Product Introduction

Citric acid (CA) is a common organic acid found in living organisms and is an important flavoring agent in food. Furthermore, CA is a product of the first step in the tricarboxylic acid cycle.

Principle

Under acidic conditions, citric acid reduces Cr^{6+} to Cr^{3+} , and Cr^{3+} has a characteristic absorption peak at 545 nm. The citric acid content in the sample can be calculated by measuring the increase in absorbance at 545 nm.

Components

Components	Size	Storage
Reagent 1	Liquid × 1 bottle	Store at 4°C
Reagent 2	Liquid × 1 bottle	Store at 4°C
Reagent 3	Liquid × 1 bottle	Store at -20°C
Reagent 4	Powder × 1 tube	RT
Reagent 5	Liquid × 1 bottle	Store at 4°C
Standard products	Liquid × 1 bottle	Store at 4°C

Storage

Store this kit according to the specified conditions upon receipt. The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

1. Citric acid extraction from liquid samples: Take 0.1 ml of liquid and add 0.9 ml of reagent, mix thoroughly, centrifuge at 11000 g, 4°C for 10 min, and take the supernatant for testing;
2. Citric acid extraction from tissue: Homogenize the tissue at a ratio of 1:5 to 10 (it is recommended to weigh about 0.1g of tissue and add 1ml of reagent one) in an ice bath, centrifuge at 11000g, 4°C for 10min, and place the supernatant on ice for analysis.
3. Citric acid extraction from mitochondria: Homogenize on ice at a ratio of tissue mass (g): extraction liquid volume (ml) of 1:5 to 10 (it is recommended to weigh about 0.1g of tissue and add 1ml of reagent one); centrifuge at 600g, 4°C for 5min; transfer the supernatant to another EP tube, centrifuge at 11000g, 4°C for 10min, and discard the supernatant; add 200μL of reagent two and 2μL of reagent three to the precipitate, suspend and dissolve thoroughly, centrifuge at 11000g, 4°C for 10min, and collect the supernatant for analysis
4. For bacteria and fungi: according to the cell number (10^4 cells): the volume of reagent I (ml) is 500-1000:1 (it is recommended to add 1 ml of reagent I for 5 million cells), sonicate the cells in an ice water bath (power 300W, sonication for 3 seconds, interval of 7 seconds, total time 3 minutes), 11000g; centrifuge at 4°C for 10 minutes, and take the supernatant for testing.

Operation process

Reagents	Blank tube	Standard tube	Test tube
Distilled water (μL)	100	--	--

Standard solution (μL)	--	100	--
Supernatant (μL)	--	--	100
Reagent 1 (μL)	700	700	700
Reagent 4 (μL)	100	100	100
Reagent 5 (μL)	100	100	100
Mix well, let stand at room temperature for 30 minutes, use a 1 mL glass cuvette, 545 nm, zero with distilled water, and measure A ₅₄₅ in each tube.			

Note: Reagent 1 needs to be preheated in a 30°C water bath for at least 30 minutes before it can be used for detection.

Result calculation

1. Calculated based on the volume of the liquid sample

$$\begin{aligned}\text{CA Content} \\ (\text{ug/mL}) &= \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times V_{\text{total}} \times \text{sample dilution factor} \\ &= 2.5 \times \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}}\end{aligned}$$

2. Calculated by organizational quality

$$\text{CA Content} \\ (\text{umol/L}) = \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times V_{\text{total}} \div W = 0.25 \times \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \div W$$

3. Calculated based on mitochondrial protein content

$$\text{CA Content} \\ (\text{umol/mgprot}) = \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times C_{\text{pr}} = 0.25 \times \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \div C_{\text{pr}}$$

4. Calculate based on cell count

$$\begin{aligned}\text{CA Content} \\ (\text{umol}/10^4\text{cell}) &= \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times V_{\text{total}} \div \text{cell count} \\ &= 0.25 \times \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \div \text{cell count}\end{aligned}$$

Precautions

1. All sample processing and other procedures must be carried out on ice.

2. Reagent 4 must be prepared and used immediately.
3. Reagent 5 contains carcinogens. Please wear gloves during the experiment to avoid splashing Reagent 5 onto your skin.
4. Citric acid extract cannot be used for protein content determination. If protein content needs to be determined, a separate tissue sample must be obtained and a protein content assay kit (BC00006) must be used.
5. This product is for research use only and must not be used for clinical diagnosis. Do not take it.