

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

Citrid Acid (CA) Colorimetric Assay Kit

Catalog No: E-BC-K351

Method: Colorimetric method

Specification: 50 Assays

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Experimental instruments

Water-bath, Refrigerate centrifuge, Spectrophotometer (545 nm), Glass cuvette (1 mL), Adjustable pipette

Detection principle

Citric acid (CA) is a common kind of organic acid in organism. In addition, CA is the first product of Krebs cycle. In acidic condition, Cr(VI) will be reduced to Cr³⁺ by citric acid. Cr³⁺ has a characteristic absorption peak at 545 nm, therefore the content of citric acid in sample can be calculated by measuring the absorbance value at 545 nm.

Reagent composition and preparation

Reagent 1: liquid, 1 vial, store at 4°C.

Reagent 2: liquid, 1 vial, store at 4°C.

Reagent 3: liquid, 1 tube, store at -20°C.

Reagent 4: powder, 1 vial, store at room temperature. Add 5 mL Reagent 1 and mix fully before use.

Reagent 5: liquid, 1 vial, store at 4°C in dark.

Standards: liquid, 1 tube, 250 μmol/L Citric Acid standard solution, store at 4°C.

Extraction of citric acid

- 1. Extraction of citric acid of liquid samples:** take 0.1 mL liquid and add 0.9 mL Reagent 1, mix fully. Centrifuge at 11000 g for 10 min at 4°C, then take the supernatant for measurement.
- 2. Extraction of citric acid of tissue sample:** add extraction solution to tissue sample in a Weight (g): Volume (mL) ratio of 1:5 ~ 1:10, make homogenization in ice water bath. Centrifuge at 11000 g for 10 min at 4°C, then take the supernatant and stand on ice for measurement.
- 3. Extraction of citric acid of mitochondria:** add extraction solution to tissue sample in a weight (g): volume (mL) ratio of 1:5 ~ 1:10, make homogenization in ice water bath. Centrifuge at 600 g for 5 min at 4°C. Take the supernatant to EP tube and centrifuge at 11000 g for 10 min at 4°C, discard the supernatant. Add 200 μL Reagent 2 and 2 μL Reagent 3, dissolve fully. Centrifuge at 11000 g for 10 min at 4°C, then take the supernatant for measurement.

Operation steps

	Blank tube	Standard tube	Sample 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 fully and stand for 30 min at room temperature. Take 1 mL cuvette, set to zero with distilled water. Measure the absorbance A_{545} of each tube at 545 nm.			

Note: The Reagent 1 should be incubated in 30°C water bath for more than 30 min before use.

Calculation of results

1. Calculate according to the volume of liquid sample

Citric acid content ($\mu\text{mol/L}$)

$$= \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} (250 \mu\text{mol/L}) \times \frac{V_{\text{Total}} (1 \text{ mL})}{V_{\text{Sample}} (0.1 \text{ mL})} \times V_{\text{Total}} (1 \text{ mL})$$

$$= \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times 2.5$$

2. Calculate according to the weight of tissue sample

Citric acid content ($\mu\text{mol/g}$)

$$= \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} (250 \mu\text{mol/L}) \times V_{\text{Total}} (1 \text{ mL}) \div W (\text{g})$$

$$= \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times 2.5 \div W (\text{g})$$

3. Calculate according to the protein content of mitochondria sample

Citric acid content ($\mu\text{mol/mg prot}$)

$$= \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} (250 \mu\text{mol/L}) \div C_{\text{Protein}} (\text{mg/mL})$$

$$= \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times 0.25 \div C_{\text{Protein}} (\text{mg/mL})$$

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.
5. The sample treatment and other procedures should be operated on ice.
6. Prepare fresh Reagent 4 before use.
7. The Reagent 5 contains carcinogenic substances, please wear gloves to protect the skin.
8. If the citric acid content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318, E-BC-K168, E-BC-K165).