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

**Catalog No：E-BC-K351-M**

**Specification：48T(32 samples)/96T(80 samples)**

**Measuring instrument：Microplate reader（535-555 nm）**

**Detection range：0.06-2.0 mmol/L**

# **Elabscience®** Citric Acid (CA) Colorimetric Assay Kit

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

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

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.

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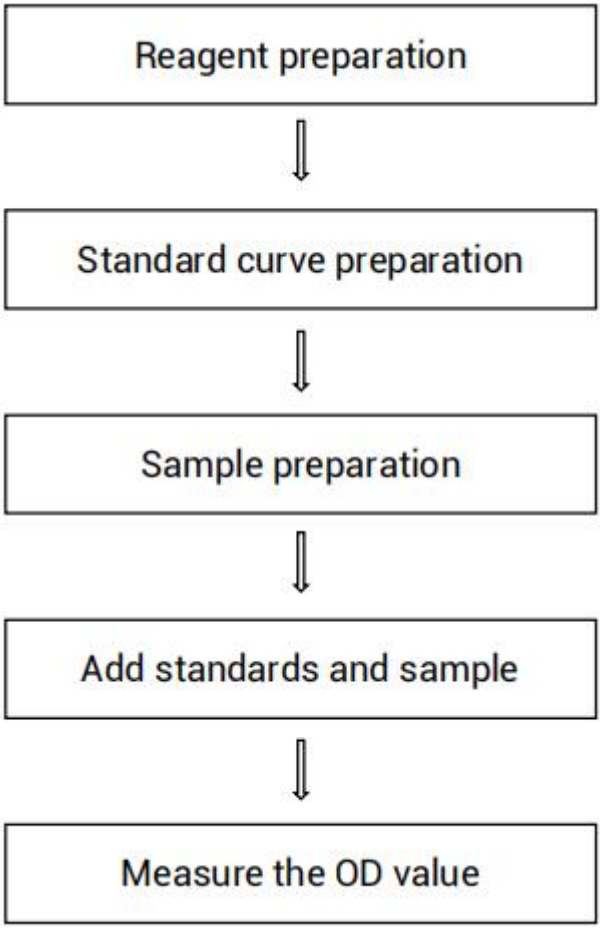
## Assay summary



## Intended use

This kit can be used to measure citric acid (CA) content in animal tissue, serum (plasma) and mitochondria samples.

## Detection principle

In acidic condition, Cr (Ⅵ) will be reduced to $Cr^{3+}$, $Cr^{3+}$ reacts with citric acid. And the product 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.

## Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Extracting Solution | 60 mL × 1 vial | 60 mL × 2 vials | 2-8°C, 12 months |
| Reagent 2 | Lysis Buffer | 10 mL × 1 vial | 20 mL × 1 vial | 2-8°C, 12 months |
| Reagent 3 | Reducing Agent | Powder × 1 vial | Powder × 1 vial | 2-8°C, 12 months shading light |
| Reagent 4 | Chromogenic Agent | 1.5 mL × 1 vial | 1.5 mL × 2 vials | 2-8°C, 12 months shading light |
| Reagent 5 | 2 mmol/L CA Standard | 2 mL × 1 vial | 2 mL × 1 vial | 2-8°C, 12 months |
| | Microplate | 48 wells | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | | |
| | Sample Layout Sheet | 1 piece | | |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

Microplate reader (535-555 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

**Reagents:**

Double distilled water

## Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② If there is solid of buffer solution, heat at 80℃ before use until clear liquid,
and cool before use.

③ The preparation of reducing agent working solution：
Dissolve a vial of reducing agent with 5 mL of double distilled water and mix fully. Aliquoted storage at -20˚C for 7 days protected from light.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 2 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 2.0, 1.5, 1.2, 1.0, 0.8, 0.5, 0.2, 0 mmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mmol/L)** | **0** | **0.2** | **0.5** | **0.8** | **1.0** | **1.2** | **1.5** | **2.0** |
| **2 mmol/L standard (μL)** | 0 | 20 | 50 | 80 | 100 | 120 | 150 | 200 |
| **Double distilled water (μL)** | 200 | 180 | 150 | 120 | 100 | 80 | 50 | 0 |

## Sample preparation

### ① Sample preparation

**Extraction of citric acid in liquid samples:** detect directly.

**Extraction of citric acid in tissue sample:**

① Take 0.1 g tissue, add 0.9 mL of extracting solution, then homogenize the sample in ice water bath.

② Centrifuge at 11000×g for 10 min at 4°C, then take the supernatant and stand on ice for measurement.

**Extraction of citric acid in mitochondria:**

① Take 0.1 g tissue, add 0.9 mL of extracting solution, then homogenize the sample in ice water bath.

② Centrifuge at 600×g for 5 min at 4°C, then take the supernatant to another EP tube and centrifuge at 10000×g for 10 min at 4°C, discard the supernatant (This supernatant can be used for the determination of citric acid content in cytoplasmic).

③ Add 200 μL of lysis buffer and dissolve fully with vortex mixer.

④ Centrifuge at 10000×g for 10 min at 4°C, then take the supernatant and stand on ice for measurement.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

### ② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|---|---|
| Human serum | 3-15 |
| Dog serum | 3-10 |
| Rat serum | 3-15 |

| | |
|---|---|
| Horse serum | 3-10 |
| Mouse plasma | 3-10 |
| 10% Rat brain tissue homogenate | 5-10 |
| 10% Rat liver tissue homogenate | 5-20 |
| 10% Rat kidney tissue homogenate | 5-10 |
| 10% Rat lung tissue homogenate | 15-30 |
| 10% Mouse heart tissue homogenate | 5-20 |

Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

## The key points of the assay

There should be no bubbles in the wells of the microplate when measuring the OD value.

## Operating steps

① Standard tube: Take 30 μL of standard solution with different concentrations into the 1.5 mL EP tubes.
Sample tube: Take 30 μL of sample into the 1.5 mL EP tubes.
② Add 210 μL of extracting solution to each tube.
③ Add 30 μL of reducing agent working solution to each tube.
④ Add 30 μL of chromogenic agent to each tube.
⑤ Mix fully and stand at room temperature for 30 min. Take 200 μL of supernatant to the microplate. Measure the OD values of each well at 545 nm with microplate reader.

## Calculation

**The standard curve**:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

**The sample**:

**1. Serum (plasma) sample:**

$$\frac{\text{CA content}}{(\text{mmol/L})} = (\triangle A - b) \div a \times f$$

**2. Tissue sample:**

$$\frac{\text{CA content}}{(\mu\text{mol/g wet weight})} = (\triangle A - b) \div a \times f \div \frac{m}{V}$$

**3. Mitochondria sample:**

$$\frac{\text{CA content}}{(\text{mmol/gprot})} = (\triangle A - b) \div a \div C_{pr} \times f$$

[Note]

ΔA: $OD_{Sample}$ - $OD_{Blank}$

f: Dilution factor of sample before test.

m: The weight of tissue sample (0.1 g).

V: The volume of extracting solution (0.9 mL).

Cpr: Protein concentration of sample (gprot/L).

# Appendix I Performance Characteristics

**1. Parameter:**

**Intra-assay Precision**

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol/L)** | 0.55 | 1.02 | 1.80 |
| **%CV** | 4.3 | 3.9 | 3.8 |

**Inter-assay Precision**

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol/L)** | 0.55 | 1.02 | 1.80 |
| **%CV** | 3.9 | 4.2 | 3.9 |

**Recovery**

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| **Expected Conc. (mmol/L)** | 0.45 | 0.85 | 1.3 |
| **Observed Conc. (mmol/L)** | 0.4 | 0.8 | 1.2 |
| **Recovery rate (%)** | 96 | 94 | 95 |

**Sensitivity**

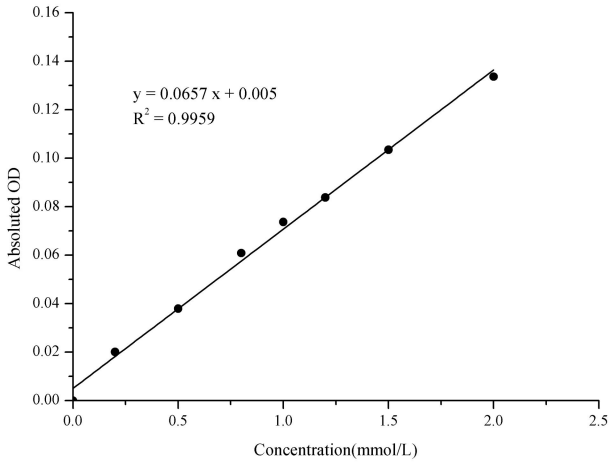
The analytical sensitivity of the assay is 0.06 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

**2. Standard curve:**

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

| Concentration (mmol/L) | 0.0 | 0.2 | 0.5 | 0.8 | 1.0 | 1.2 | 1.5 | 2.0 |
|---|---|---|---|---|---|---|---|---|
| OD value | 0.071 | 0.091 | 0.112 | 0.136 | 0.147 | 0.154 | 0.177 | 0.204 |
| | 0.071 | 0.091 | 0.106 | 0.127 | 0.142 | 0.156 | 0.172 | 0.205 |
| Average OD | 0.071 | 0.091 | 0.109 | 0.132 | 0.145 | 0.155 | 0.174 | 0.205 |
| Absoluted OD | 0.000 | 0.020 | 0.038 | 0.061 | 0.074 | 0.084 | 0.103 | 0.134 |



## Appendix Π Example Analysis

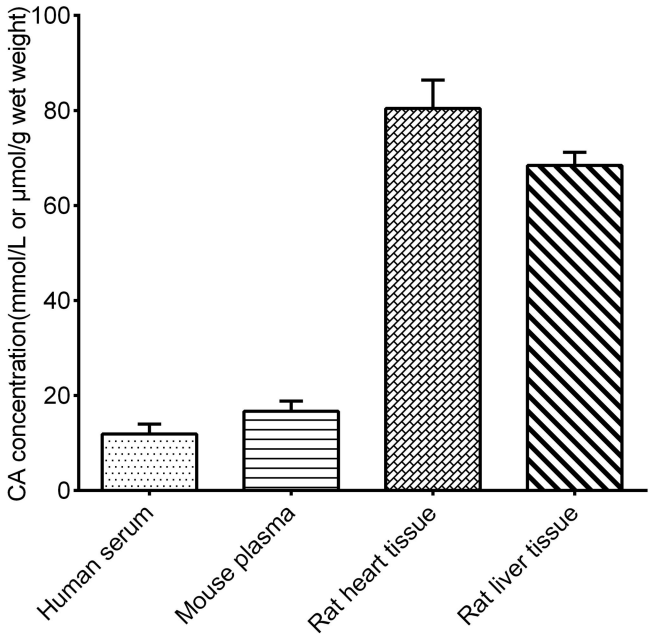
**Example analysis：**

Take 0.1 mL of human serum and add 0.9 mL of extracting solution, mix fully and extracted by vortex for 1 min. Take 30 μL to the corresponding tubes and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0672 x + 0.0009, the OD value of the sample is 0.142, the OD value of the blank is 0.071, and the calculation result is:

$$\text{CA content (mmol/L)} = (0.142 - 0.071 - 0.0009) \div 0.0672 \times 10 = 10.432 \text{ mmol/L}$$

Detect human serum (dilute for 10 times), mouse plasma (dilute for 10 times), 10% rat heart tissue homogenate (dilute for 20 times) and 10% rat liver tissue homogenate (dilute for 20 times) according to the protocol, the result is as follows：



## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.