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

Catalog No：E-BC-K351-S

Specification：50Assays(48 samples)/100Assays (96 samples)

Measuring instrument：Spectrophotometer（545 nm）

Detection range：0.05-5.0 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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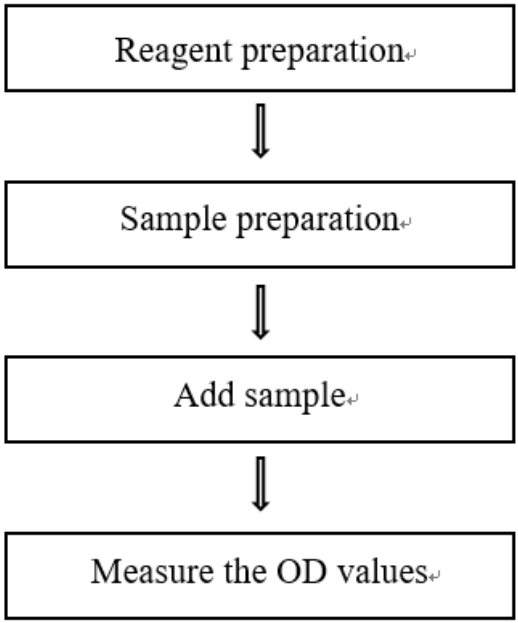
Assay summary



Intended use

This kit can be used to measure citric acid (CA) content in tissue, mitochondria and other liquid 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	45 mL ×2 vials	45 mL ×4 vials	2-8℃, 12 months
Reagent 2	Lysis Buffer	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months
Reagent 3	Reducing Agent	Powder ×1 vial	Powder ×1 vial	2-8℃, 12 months shading light
Reagent 4	Chromogenic Agent	10 mL ×1 vial	15 mL ×1 vial	2-8℃, 12 months shading light
Reagent 5	1 mmol/L CA Standard	2 mL ×1 vial	2 mL ×1 vial	2-8℃, 12 months

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:

Spectrophotometer (545 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water

Reagent preparation

① If there is solid of buffer solution, heat at 80℃ before use until clear liquid, and cool before use.

② The preparation of reducing working solution：

50Assays: Dissolve a vial of reducing agent with 10 mL buffer solution and mix fully. Store at 2~8 ℃ for 7 days.

100Assays: Dissolve a vial of reducing agent with 20 mL buffer solution and mix fully. Store at 2~8 ℃ for 7 days.

③ The preparation of 0.25 mmol/L standard application solution：

For each tube, prepare 100 μL of 0.25 mmol/L standard application solution (mix well 25 μL of 1 mmol/L CA standard and 75 μL of double distilled water). The 0.25 mmol/L standard application solution should be prepared on spot.

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 buffer solution, then homogenize the sample in ice water bath.

② Centrifuge at 11000×g for 10 min at 4℃, 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 buffer solution, then homogenize the sample in ice water bath.

② Centrifuge at 600 g for 5 min at 4℃, then take the supernatant to another EP tube and centrifuge at 11000×g for 10 min at 4℃, 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 11000×g for 10 min at 4℃, 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	4-6
10% Mouse kidney tissue homogenate	2-4
10% Rat kidney tissue homogenate	1
10% Mouse heart tissue homogenate	2-4
10% Mouse brain tissue homogenate	1
10% Mouse liver tissue homogenate	1

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

The key points of the assay

Preheat buffer solution at 30 ℃ water bath for 30 min before use.

Operating steps

① Blank tube: Take 100 μL of double distilled water to the 1.5 mL EP tube.
Standard tube: Take 100 μL of 0.25 mmol/L standard application solution to the 1.5 mL EP tube.
Sample tube: Take 100 μL of sample supernatant in sample preparation step to the 1.5 mL EP tube.

② Add 700 μL of buffer solution to each tube.

③ Add 100 μL of reducing agent working solution to each tube.

④ Add 100 μL of chromogenic agent to each tube

⑤ Mix fully with vortex mixer and stand for 30 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 545 nm wavelength with 1 mL quartz cuvette.

Calculation

The sample:

1. Liquid sample:

$$\text{CA content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

$$\text{CA content (μmol/g fresh weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

3. Mitochondria sample:

$$\text{CA content (μmol/mg prot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

ΔA_1: OD_{Sample} - OD_{Blank}

ΔA_2: $OD_{Standard}$ - OD_{Blank}

c: Concentration of standard (0.25 mmol/L).

f: Dilution factor of sample before test.

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

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

C_{pr}: Protein concentration of sample (mgprot/mL).

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)	0.85	1.50	3.40
%CV	4.6	4.1	3.9

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)	0.85	1.50	3.40
%CV	5.2	5.7	5.3

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 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	1.2	2.9	4.3
Observed Conc. (mmol/L)	1.2	2.7	4.1
Recovery rate (%)	99	94	95

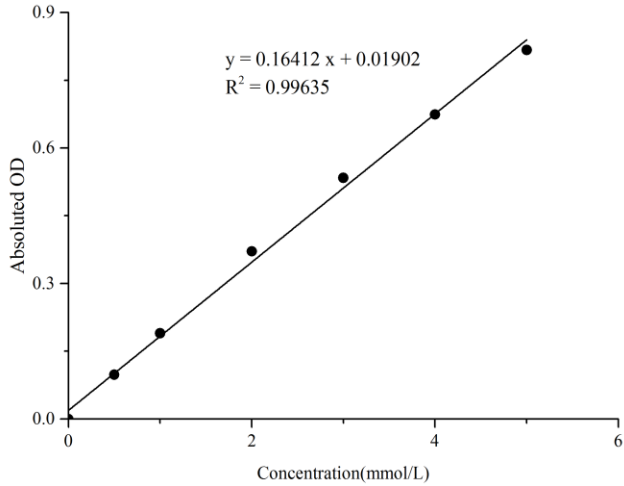
Sensitivity

The analytical sensitivity of the assay is 0.05 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**	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**
OD value	0.101	0.202	0.288	0.466	0.580	0.785	0.909
	0.105	0.200	0.297	0.481	0.694	0.769	0.931
Average OD	0.103	0.201	0.293	0.474	0.637	0.777	0.920
Absoluted OD	0	0.098	0.190	0.371	0.534	0.674	0.817



Appendix II Example Analysis

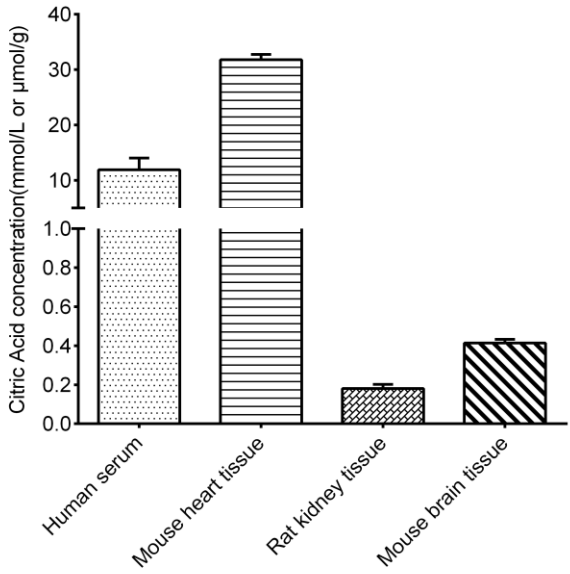
Example analysis：

Dilute human serum with buffer solution for 5 times, take 100 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.556, the average OD value of the blank is 0.119, the average OD value of the standard is 0.173, and the calculation result is:

CA content (mmol/L) = (0.556 - 0.119) ÷ (0.173 - 0.119) × 0.25 × 5 = 10.116 mmol/L

Detect human serum (dilute for 5 times), 10% mouse heart tissue homogenate (dilute for 3 times), 10% rat kidney tissue homogenate and 10% mouse brain tissue homogenate 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.