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

Catalog No：E-BC-F153

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.81-100 μmol/L

Elabscience® Citrate Fluorometric 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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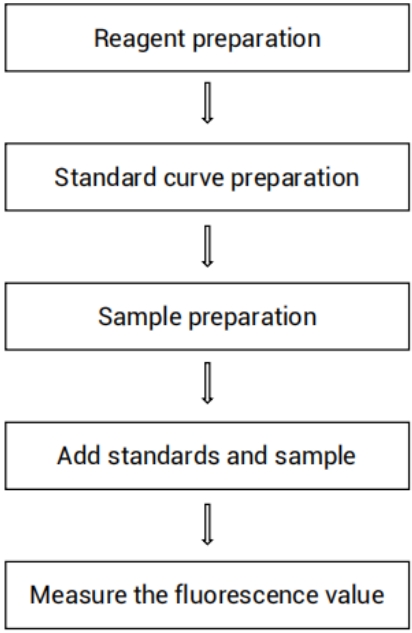
Assay summary



Intended use

The kit is suitable for detecting the content of citrate in animal tissues and cells.

Detection principle

Citrate is widely present in animal blood, tissues, and plant fruits. In living organisms, citrate is typically synthesized from oxaloacetate and acetyl-CoA via citrate synthase. As an intermediate metabolite in the tricarboxylic acid (TCA) cycle, CA can be rapidly utilized to generate ATP under stress conditions, thereby enhancing the organism's resistance to stress.

Citrate is converted into oxaloacetate under the catalysis of citrate lyase. Oxaloacetate is subsequently transformed into hydrogen peroxide (H_2O_2) via multiple enzymatic reactions. The generated H_2O_2 reacts with the fluorescent probe ADHP to produce a fluorescent substance. The fluorescence intensity can be measured to calculate the citrate content in the sample.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	46 mL × 1 vial	46 mL × 2 vials	-20°C, 12 months
Reagent 2	Cofactor	Power × 1 vial	Power × 2 vials	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent A	0.055 mL × 1 vial	0.055 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Enzyme Reagent B	Power × 1 vial	Power × 2 vials	-20°C, 12 months, shading light
Reagent 5	Enzyme Reagent C	0.055 mL × 1 vial	0.055 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Probe	0.055 mL × 1 vial	0.055 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	10 mmol/L Standard	0.18 mL × 1 vial	0.36 mL × 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator,

Materials required but not provided

Distilled or deionized water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), 10 kDa MWCO Spin Filter (Outer tube 1.5 mL, Inter tube 0.5 mL)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Cofactor Working Solution preparation:
Dissolve one vial of Cofactor with 550 μL of distilled or deionized water. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.

③ Enzyme Reagent B Working Solution preparation:
Dissolve one vial of Cofactor with 275 μL of distilled or deionized water. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.

④ Working Solution preparation:
Before testing, please prepare sufficient Working Solution. For example, prepare 626 μL of Working Solution (mix well 610 μL of Buffer Solution, 50 μL of Cofactor Working Solution preparation, 5 μL of Enzyme Reagent A, 25 μL Enzyme Reagent B Working Solution and 5 μL of Enzyme Reagent C). The Working Solution should be freshly prepared before use. Stable for 2 h protected from light.

⑤ Chromogenic Working Solution preparation：
Before testing, prepare a sufficient Chromogenic Working Solution according to the test wells. For example, prepare 255 μL of Chromogenic Working Solution (mix 250 μL of Buffer Solution and 5 μL of Probe thoroughly). Stable for 1 h when stored at -20°C protected from light.

⑥ 100 μmol/L Standard Solution preparation:

Before testing, prepare a sufficient 100 μmol/L Standard Solution according to the test wells. For example, prepare 1000 μL of 100 μmol/L Standard Solution (mix 990 μL of distilled or deionized water and 10 μL of 10 mmol/L Standard thoroughly). Stable for 3 days when stored at -20°C protected from light.

⑦ Standard curve preparation：

Always prepare a fresh set of standards. Discard Working Standard Dilutions after use.

Dilute 100 μmol/L Standard Solution with distilled or deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 200, 40, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**40**	**60**	**80**	**100**
100 μmol/L Standard (μL)	0	10	20	40	80	120	160	200
distilled or deionized water (μL)	200	190	180	160	120	80	40	0

Sample preparation

Serum or plasma samples:

① Add serum or plasma sample into 10kDa MWCO Spin Filter and centrifuge at 12000×g for 25 min.

② Collect the filtrate from the lower filter tube and preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg). Wash tissue in cold PBS (0.01 M, pH 7.4).

② Homogenize 50 mg tissue in 450 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Transfer the supernatant to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection.

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells). Wash cells with PBS (0.01 M, pH 7.4).

② Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a dounce homogenizer.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Transfer the supernatant to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection.

Dilution of sample

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

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse spleen tissue homogenization	1
10% Mouse lung tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Orange pulp tissue homogenization	5-40
10% Orange peel tissue homogenization	2-10
Human serum	2-20
Rat serum	2-5
Pig serum	1
Horse serum	1
Human milk	8-20
1×10^6 293T cells	1
1×10^6 RAW 264.7 cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of Standard Solution with different concentrations to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.

② Add 120 μL of Working Solution into each well.

③ Add 50 μL of Chromogenic Working Solution into each well.

④ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 37°C for 30 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using corrected fluorescence 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 or plasma samples:

$$\text{citrate content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{citrate content (μmol/kg wet weight)} = (\Delta F - b) \div a \times V \div m \times f$$

3. Cell sample:

$$\text{citrate content (nmol/10^9)} = (\Delta F - b) \div a \times V \div n \times f \times 1000$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{blank}$

m: The weight of tissue, kg.

V: The volume of normal saline (0.9% NaCl).

n: The number of cell sample/10^9.

f: Dilution factor of sample before tested.

1000: 1 μmol/L=1000 nmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three orange pulp 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 (μmol/L)	15	48	91
%CV	1.3	0.9	0.8

Inter-assay Precision

Three orange pulp samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15	48	91
%CV	3.2	1.1	5.8

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 103.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	15	48	91
Observed Conc. (μmol/L)	15.45	49.44	95.55
Recovery rate (%)	103	103	105

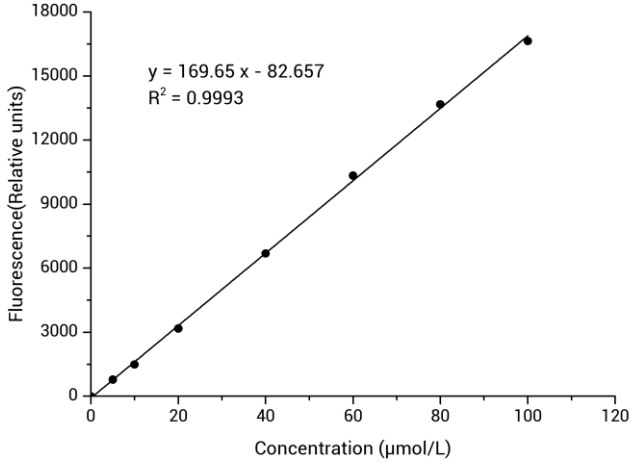
Sensitivity

The analytical sensitivity of the assay is 0.81 μmol/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 fluorescence 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 (μmol/L)	**0**	**5**	**10**	**20**	**40**	**60**	**80**	**100**
Fluorescene value	3550	4328	5068	6736	10283	13977	17097	20138
	3558	4346	5024	6718	10199	13795	17353	20254
Average fluorescene value	3554	4337	5046	6727	10241	13886	17225	20196
Corrected fluorescene value	0	783	1492	3173	6687	10332	20196	16642



Appendix Π Example Analysis

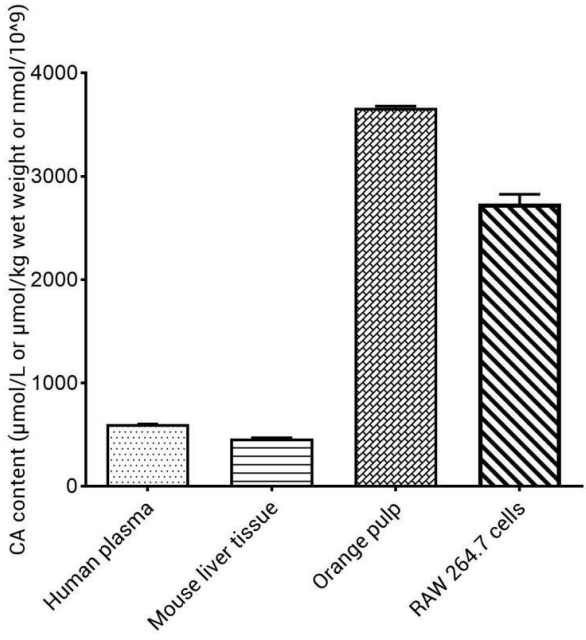
Example analysis：

Take 20 μL of 10% mouse liver tissue filtrate to the well of microplate. Proceed according to the operating steps. The results are as follows: standard curve: y = 169.65 x - 82.657, the average fluorescence value of the blank well is 3554 the average fluorescence value of the sample well is 12241, $\Delta F = F_{sample} - F_{blank}$ = 12241 - 3554 = 8687, and the calculation result is:

citrate content (μmol/kg wet weight) = (8687+82.657) ÷ 169.65 × 0.0009 ÷ 0.0001

= 465.23 μmol/kg wet weight

Detect human serum (dilute for 7 times), 10% mouse liver tissue homogenate supernatant, 10% orange pulp tissue homogenate supernatant and 1×10^6 RAW 264.7 cells 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.