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

Catalog No：E-BC-K1106-M

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

Measuring instrument：Microplate reader (545-555 nm)

Detection range：0.006-1 mmol/L

Elabscience® Oxalate (Oxalic Acid) Colorimetric Assay Kit (Extraction method)

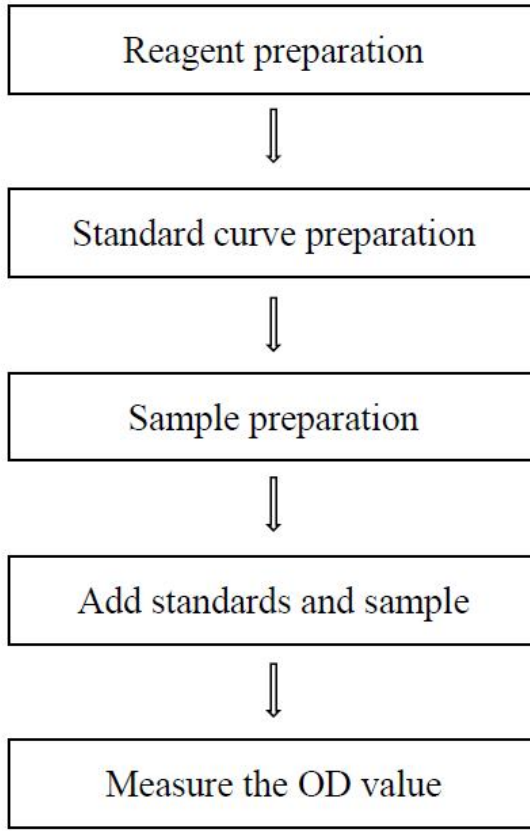
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure the oxalate (oxalic acid) content in plasma (serum), urine, animal and plant tissue samples.

Detection principle

Oxalic, also known as ethanedioic acid, is a simple organic acid in plants and plays important physiological roles. Oxalic participates in the regulation of Ca^{2+}, pH and osmotic pressure within cells, as well as the defense responses of plant pathogens and the alleviation of aluminum toxicity. Although Oxalic has important physiological functions, excessive intake of Oxalic can also cause many adverse effects on the human body. Oxalic affects the absorption of mineral elements such as calcium and iron by the human body, increases the risk of developing kidney stones, inflammation, hyperoxaluria, uremia and other diseases, and in severe cases, it can even cause acute Oxalic poisoning. Moreover, oxalic is not easily oxidized and decomposed in the human body, and its metabolites can disrupt the acid-base balance in the body.

The detection principle of this kit: Oxalate oxidase catalyzes the oxidation of oxalate to produce hydrogen peroxide and carbon dioxide. Hydrogen peroxide reacts with chromogenic substances under the action of enzymes to form colored products. There is a maximum absorption peak at 550 nm, and the color depth is proportional to the content of oxalic.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	40 mL × 1 vial	40 mL × 2 vials	-20℃, 12 months
Reagent 2	Chromogenic Agent A	3 mL × 1 vial	6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Chromogenic Agent B	3 mL × 1 vial	6 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Enzyme Reagent A	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 5	Enzyme Reagent B	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 6	Precipitator	12 mL × 1 vial	24 mL × 1 vial	-20℃, 12 months
Reagent 7	Acid Solution	12 mL × 1 vial	24 mL × 1 vial	-20℃, 12 months
Reagent 8	Alkali Solution	6 mL × 1 vial	12 mL × 1 vial	-20℃, 12 months
Reagent 9	1 mmol/L Standard Solution	1.8 mL × 1 vial	1.8 mL× 1 vial	-20℃, 12 months, shading light
	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 (545-555 nm, optimum wavelength: 550 nm), Incubator (37°C)

Reagents:

Absolute ethanol

Consumptive material:

10kDa MWCO Spin Filter (Outer tube 1.5 mL; Inner tube 0.5 mL), pH test paper

Reagent preparation

① Equilibrate all reagents to 25°C before use, check if there is any precipitation in precipitator, heat it at 37°C until it dissolves completely.

② The preparation of enzyme reagent A working solution：
Dissolve one vial of enzyme reagent A with 6 mL of buffer solution, mix fully and keep the solution on ice during use. Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of enzyme reagent B working solution：
Dissolve one vial of enzyme reagent B with 0.2 mL of double distilled water, mix fully. Aliquoted storage at -20°C for 7 days protected from light.

④ The preparation of chromogenic working solution：
Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 510 μL of chromogenic working solution (mix well 250 μL of chromogenic agent A, 250 μL of chromogenic agent B and 10 μL of enzyme reagent B working solution). The chromogenic working solution should be prepared on spot and the prepared solution should be used up within 1 h protected from

light.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**1.0**
1 mmol/L standard (μL)	0	40	60	80	100	120	140	200
Buffer Solution (μL)	200	160	140	120	100	80	60	0

Sample preparation

① **Sample preparation**

Serum and plasma: Centrifuge with 10kDa MWCO spin filter at 12000×g for 10 min. Take the filtrate for sample pretreatment.

Urine: Conduct the sample pretreatment directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4°C.

③ Centrifuge at 12000 × g for 10 min at 4°C. Collect supernatant and keep it on ice for sample pretreatment.

Sample pretreatment:

① Take 400 μL of sample, add 200 μL of precipitator and 800 μL of absolute ethanol, mix fully and stand at 2-8°C for 1 h.

② Centrifuge at 15000 × g for 10 min to remove supernatant. Add 1500 μL of double distilled water, mix fully and centrifuge at 15000 × g for 10 min.

③ Remove the supernatant, add 100 μL of acid solution, mix fully until the precipitate is completely dissolved.

④ Take 50 μL of solution to 2 mL EP tube, add 460 μL of buffer solution and 90 μL of alkali solution, mix well and keep it at room temperature for use. (If the solution turns from clear to cloudy after adding the alkali solution, it is recommended to add a small amount (5-20 μL) of acid solution in several steps and observe when the solution becomes clear. Then stop adding acid solution and measure the pH of the solution within the range of 4-6).

The processed samples are valid for testing on the same day.

② **Dilution of sample**

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Spinach tissue homogenate	1
10% Pumpkin tissue homogenate	1
Human urine	1
Rat urine	1
Human serum	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

Sample pretreatment of ②, the pipette tip was used to fully suspend the precipitate to make it evenly distributed.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the standard wells.

Sample well: add 20 μL of sample into the sample wells.

② Add 100 μL of enzyme reagent A working solution into each well.

③ Incubate at 37°C for 10 min protected from light.

④ Add 100 μL of chromogenic working solution into each well.

⑤ Mix well with microplate reader for 5 s. Incubate at 37°C for 10 min protected from light. Measure the OD values of each well at 550 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. Plasma, serum and urine sample:

$$\text{Oxalate content (mmol/L)} = (\Delta A - b) \div a \times f \times 3^*$$

2. Tissue sample:

$$\text{Oxalate content (mmol/kg wet weight)} = (\Delta A - b) \div a \div \frac{m}{v} \times f \times 3^*$$

[Note]

ΔA: $A_{sample} - A_{blank}$

v: The volume of sample homogenate, mL

m: The weight of the sample, g.

f: Dilution factor of sample before test.

3*: Dilution factor of sample during sample pretreatment.

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)	0.10	0.50	0.80
%CV	2.1	3.2	2.4

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.10	0.50	0.80
%CV	4.5	7.2	6.3

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 98.7%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.10	0.50	0.80
Observed Conc. (mmol/L)	0.10	0.50	0.82
Recovery rate (%)	98	100	98

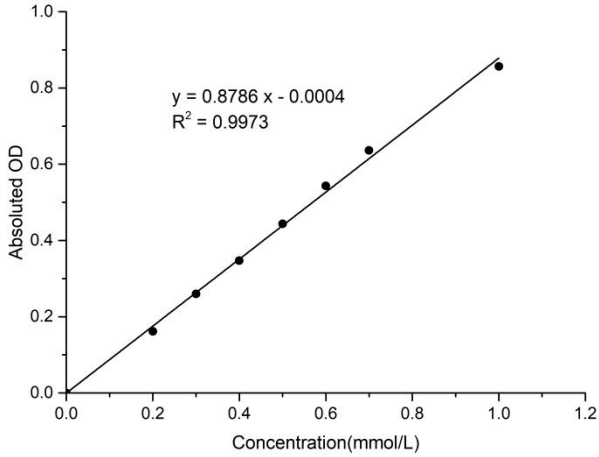
Sensitivity

The analytical sensitivity of the assay is 0.006 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	0.2	0.3	0.4	0.5	0.6	0.7	1.0
OD	0.042	0.211	0.302	0.392	0.502	0.578	0.682	0.926
	0.043	0.197	0.303	0.387	0.470	0.593	0.675	0.872
Average OD	0.043	0.204	0.303	0.390	0.486	0.586	0.679	0.899
Absoluted OD	0.000	0.162	0.260	0.347	0.444	0.543	0.636	0.857



Appendix Π Example Analysis

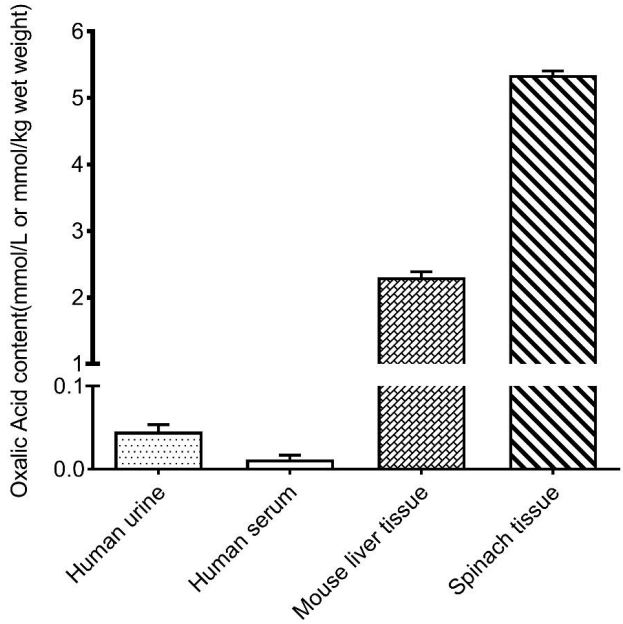
Example analysis：

Take 20 μL of human urine sample, and carry the assay according to the operation steps. The results are as follows:

The standard curve: y = 0.8786 x - 0.0004, the OD value of the sample well is 0.055, the OD value of the blank well is 0.043, and the calculation result is:

$$\text{Oxalate content}_{(\text{mmol/L})} = (0.055 - 0.043 + 0.0004) \div 0.8786 \times 3 = 0.042 \text{ mmol/L}$$

Detect human urine, human serum, 10% mouse liver tissue homogenate and 10% spinach 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.