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

Catalog No：E-BC-K892-S

Specification：50 Assays (34 samples)/100 Assays (84 samples)

Measuring instrument：Spectrophotometer (550 nm)

Detection range：0.08-1.00 mmol/L

Elabscience® Oxalate 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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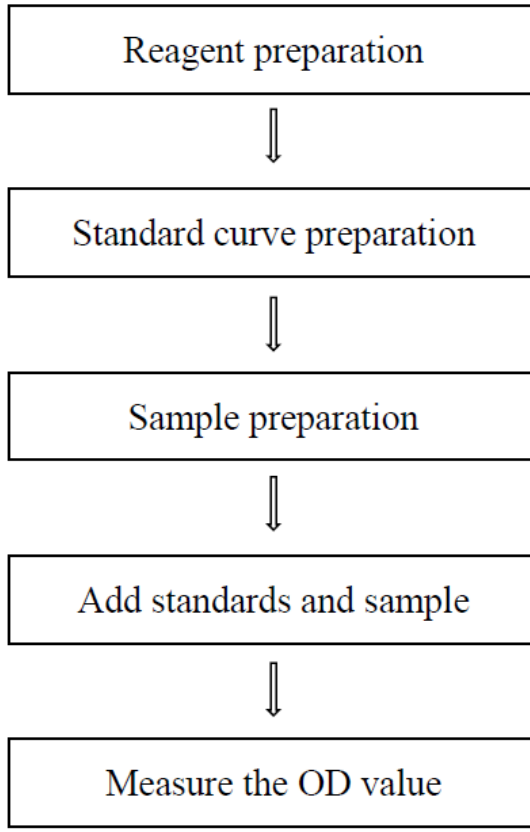
Assay summary



Intended use

This kit can be used to measure total oxalate content in serum, plasma, urine, plant and animal tissue samples.

Detection principle

Oxalic acid is a simple organic acid in organisms and has important physiological functions. The detection principle of this kit: Oxalate oxidase catalyzes the oxidation of oxalate to produce hydrogen peroxide and carbon dioxide. Under the action of HRP, hydrogen peroxide reacts with chromogenic substances to produce colored products. There is a maximum absorption peak at 550 nm, and the absorbance is proportional to the content of oxalate.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	50 mL ×1 vial	50 mL ×2 vials	-20℃, 12 months
Reagent 2	Chromogenic Agent A	15 mL ×1 vial	30 mL ×1 vial	-20℃, 12 months shading light
Reagent 3	Chromogenic Agent B	15 mL ×1 vial	30 mL ×1 vial	-20℃, 12 months shading light
Reagent 4	Enzyme Reagent A	Powder ×2 vials	Powder ×4 vials	-20℃, 12 months shading light
Reagent 5	Enzyme Reagent B	Powder ×2 vials	Powder ×4 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	2 mL ×1 vial	2 mL ×1 vial	-20℃, 12 months shading light

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 (550 nm), Incubator (37℃)

Consumptive material:

10kDa MWCO Spin Filter

Reagents:

Absolute ethanol

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② Preparation of enzyme reagent A working solution：
Dissolve one vial of enzyme reagent A with 15 mL of buffer solution, mix well to dissolve. Keep it on ice during use. Aliquoted storage at -20 ℃ for 7 days protected from light.

③ Preparation of enzyme reagent B working solution：
Dissolve one vial of enzyme reagent B with 0.5 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20 ℃ for 7 days protected from light.

④ 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 prepared solution should be prepared on spot and 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 with double distilled water 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
Double distilled water (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Serum and plasma: Add serum (plasma) sample into 10kDa MWCO Spin Filter and centrifuge at 12000×g for 10 min. Collect the filtrate and prepare for sample pretreatment.

Urine sample: Use directly for sample pretreatment.

Tissue sample:

① Harvest the amount of plant tissue needed for each assay (initial recommendation 100 mg).

② Homogenize 100 mg tissue in 900 μL buffer solution with a dounce homogenizer at 4 ℃.

③ Centrifuge at 12000×g for 10 min at 4 ℃ to remove insoluble material. Collect the supernatant and prepare for sample pretreatment.

The sample pretreatment:

① Add 400 μL of sample, 200 μL of precipitator and 800 μL of absolute ethanol into 2 mL EP tube. Mix well and stand for 1 h at 2-8℃.

② Centrifuge at 15000×g for 10 min to remove supernatant. Add 1500 μL of double distilled water and suspend the precipitate fully with the micropipettor tip. Centrifuge at 15000×g for 10 min at 4 ℃.

③ Remove supernatant and add 100 μL of acid solution. Dissolve completely to get solution A.

④ Take 50 μL of solution A into a new 2 mL EP tube. Add 460 μL of buffer solution and 90 μL of alkali solution. Mix well and preserve it on room temperature for detection.

Note: Detect the prepared sample 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
Rats plasma	1
Human plasma	1
Human urine	1
10% Epipremnum aureum tissue homogenate	2-3
10% Spinach tissue homogenate	1
10% Pumpkin 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

During sample pretreatment, the sample pretreatment steps should be strictly followed, and the supernatant and precipitate should be carefully absorbed during processing.

Operating steps

① Standard tube: Add 100 μL of standard with different concentrations to 2 mL EP tube.

Sample tube: Add 100 μL of sample to 2 mL EP tube.

② Add 500 μL of enzyme reagent A working solution into each tube.

③ Incubate at 37 ℃ for 10 min protected from light.

④ Add 500 μL of chromogenic working solution into each tube.

⑤ Incubate at 37 ℃ for 10 min protected from light.

⑥ Set the spectrophotometer to zero with blank tube (Standard tube with a standard concentration of 0 mmol/L) and measure the absorbance values of each tube at 550 nm with 0.5 cm optical path cuvette.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Plot the standard curve by using 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. Liquid sample:

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

2. Tissue sample:

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

[Note]

A: The OD value of the sample.

3*: Dilution factor of sample in sample pretreatment step.

m: The weight of sample, g.

V: The volume of sample homogenate, mL.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human plasma 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	1.80	2.00	2.05

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)	0.15	0.45	0.80
%CV	4.50	5.00	7.20

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)	0.15	0.50	0.80
Observed Conc. (mmol/L)	0.147	0.500	0.785
Recovery rate (%)	98	100	98.2

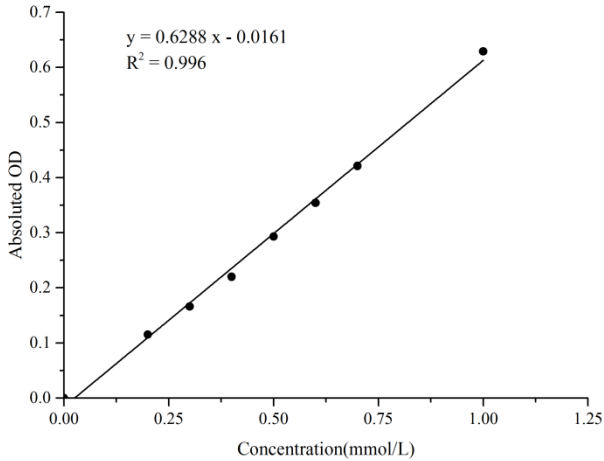
Sensitivity

The analytical sensitivity of the assay is 0.08 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 value	0.000	0.115	0.166	0.220	0.293	0.354	0.421	0.629



Appendix II Example Analysis

Example analysis:

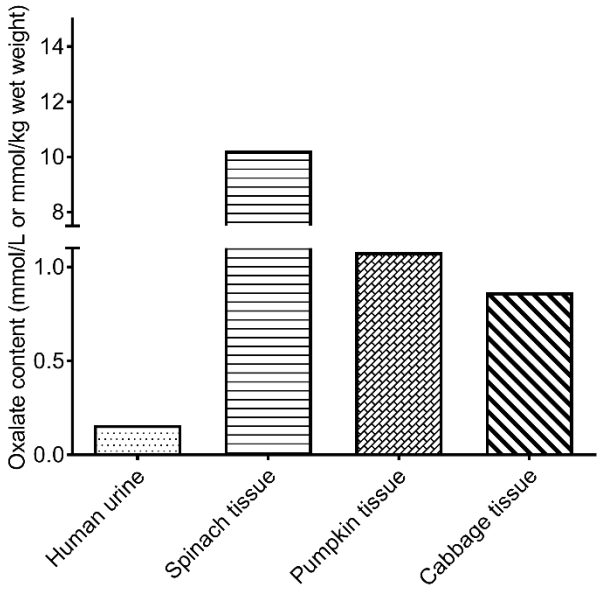
Take 100 μL of 10% spinach tissue of pretreatment solution and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.6288 x - 0.0161, the OD value of the sample tube is 0.222, and the calculation result is:

Oxalate content (mmol/kg wet weight) = （0.222 + 0.0161）÷0.6288 ÷(0.1 ÷0.9) ×3

= 10.224 mmol/kg wet weight

Detect human urine, 10% spinach tissue homogenate, 10% pumpkin tissue homogenate and 10% cabbage 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.