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

Catalog No：E-BC-K892-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（545-555 nm）

Detection range：0.02-1 mmol/L

Elabscience®Oxalate (Oxalic Acid) 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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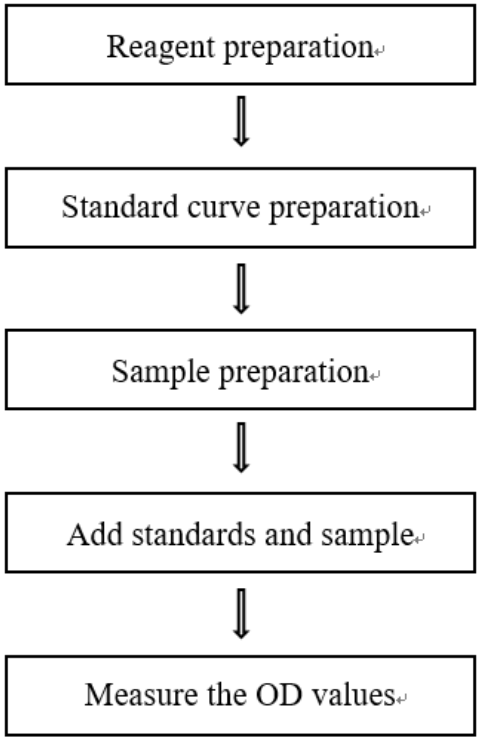
Assay summary



Intended use

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

Detection principle

Oxalate oxidase catalyzes the oxidation of oxalate to produce hydrogen peroxide and carbon dioxide. Under the action of POD, hydrogen peroxide reacts with chromogenic substances to produce colored products. There is a specific absorption peak at 550 nm, and the color depth is proportional to the content of oxalate.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Working Solution	12 mL ×1 vial	-20℃, 12 months
Reagent 2	Chromogenic Agent A	12 mL ×1 vial	-20℃, 12 months shading light
Reagent 3	Chromogenic Agent B	12 mL ×1 vial	-20℃, 12 months shading light
Reagent 4	Enzyme Reagent A	Powder ×2 vials	-20℃, 12 months
Reagent 5	Enzyme Reagent B	Powder ×2 vials	-20℃, 12 months
Reagent 6	Regulator	12 mL ×1 vial	-20℃, 12 months
Reagent 7	Clarificant	2 mL ×1 vial	-20℃, 12 months
Reagent 8	1 mmol/L Standard	2 mL ×1 vial	-20℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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:

Micropipettor, Microplate reader (545-555 nm, optimum wavelength: 550 nm), Centrifuge, 37℃ incubator

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of enzyme reagent A working solution:
Dissolve one vial of enzyme reagent A with 5 mL of working solution, mix well to dissolve. Keep the prepared solution on ice during use. Store at 2-8 ℃ for 2 days.

③ Preparation of enzyme reagent B working solution:
Dissolve one vial of enzyme reagent B with 250 μL of double distilled water, mix well to dissolve. Store at 2-8 ℃ for 2 days.

④ Preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 201 μL of reaction working solution (mix well 100 μL of chromogenic agent A, 100 μL of chromogenic agent B and 1 μL of enzyme reagent B working solution). The prepared solution should be prepared on spot and used up within 1 h.

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, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**1.0**
1 mmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Urine: For each well， add 10 μL of sample and 10 μL of regulator, mix well. Stand at room temperature for 10 min for detection.

Plant tissue sample:

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

② Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4 ℃.

③ Centrifuge at 10000×g for 10 min at 4 ℃ to remove insoluble material. Collect supernatant.

④ For each well，add 10 μL of supernatant and 10 μL of regulator, mix well. Stand at room temperature for 10 min for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human urine	1-2
Human plasma	1
10% Epipremnum aureum tissue homogenate	2-3
Rats plasma	1

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

The key points of the assay

① When detecting urine samples, add regulators as required for sample preparation.

② Adjust the dilution ratio of samples according to the pre-experiment results.

③ Pay attention to the storage conditions when using enzyme reagents..

Operating steps

① Sample well: Add 10 μL of sample to corresponding well.
Control well: Add 10 μL of sample to corresponding well.
Standard well: Add 10 μL of standard with different concentrations to corresponding well.

② Add 80 μL of enzyme reagent A working solution into sample well and standard well. Add 80 μL of double distilled water into control well.

③ Incubate at 37 ℃ for 10 min.

④ Add 120 μL of reaction working solution into each well.

⑤ Stand at room temperature for 2 min.

⑥ Add 20 μL of clarificant into each well.

⑦ Mix fully with microplate reader 5 s and measure the OD value 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. Urine sample:

$$\text{Oxalate content (mmol/L)} = (\Delta A - b) \div a \times 2^* \times f$$

2. Serum/plasma sample:

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

3. Plant tissue sample:

$$\text{Oxalate content (mmol/ kg wet weight)} = (\Delta A - b) \div a \div (m \div V) \times 2^* \times f$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

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

m: the weight of sample, 0.1 g.

V: the volume of sample homogenate, 0.9 mL.

f: Dilution factor of sample before tested

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）	0.08	0.54	0.88
%CV	3.5	3.1	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）	0.08	0.54	0.88
%CV	4.8	5.2	4.1

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）	0.35	0.55	0.75
Observed Conc. (mmol/L）	0.3	0.5	0.7
Recovery rate (%)	96	94	95

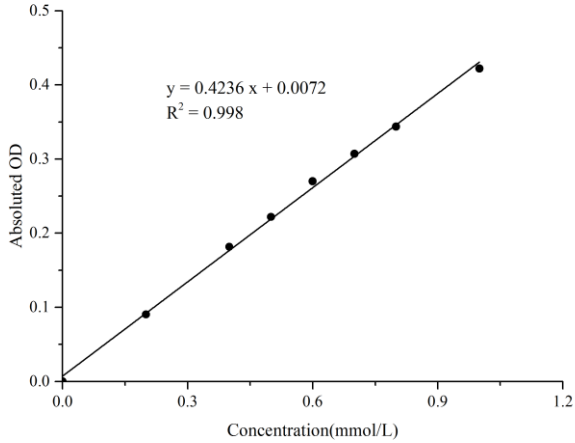
Sensitivity

The analytical sensitivity of the assay is 0.02 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**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**1.0**
Average OD	0.046	0.137	0.228	0.268	0.316	0.353	0.390	0.468
Absoluted OD	0.000	0.090	0.182	0.222	0.270	0.307	0.344	0.422



Appendix II Example Analysis

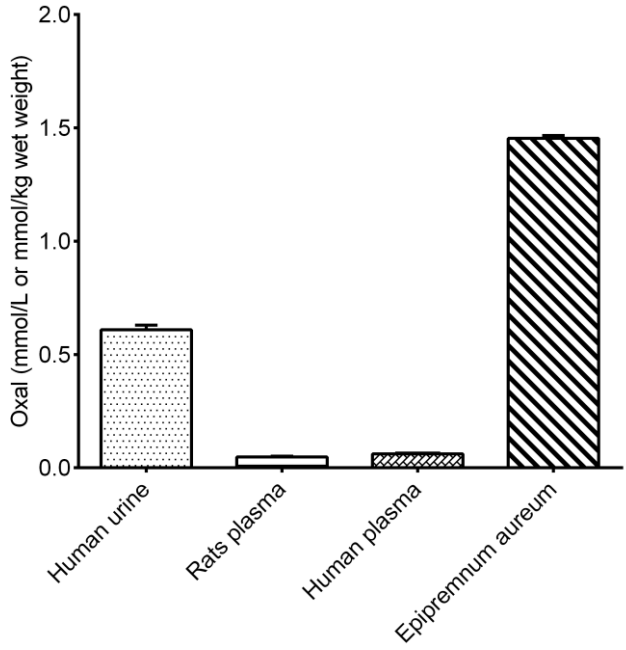
Example analysis:

For human urine, take 10 μL of human urine and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.4236x + 0.0072, the average OD value of the sample is 0.352, the average OD value of the control is 0.216, and the calculation result is:

Oxalate content (mmol/L) =（0.352-0.216-0.0072）÷0.4236×2=0.60 mmol/L

Detect human urine, rats plasma, human plasma, 10% epipremnum aureum 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.