TECHNICAL MANUAL

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

- **SKU CODE:** MAES0512
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Sample pretreatment
- Add standards and samples
- Add enzyme reagent and incubate
- Add chromogenic working solution
- Measure absorbance at 550 nm

2. Intended use

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

3. Detection principle

Oxalic acid, also known as ethanedioic acid, is a simple organic acid in plants that plays important physiological roles. Oxalic acid participates in the regulation of Ca2+, pH and osmotic pressure within cells, as well as defense responses to plant pathogens and alleviation of aluminum toxicity. Although oxalic acid has important physiological functions, excessive intake can cause adverse effects on the human body. Oxalic acid affects the absorption of mineral elements such as calcium and iron, increases the risk of developing kidney stones, inflammation, hyperoxaluria, uremia and other diseases, and in severe cases, can even cause acute oxalic acid poisoning. Moreover, oxalic acid is not easily oxidized and decomposed in the human body, and its metabolites can disrupt the acid-base balance.

Detection principle: 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 oxalic acid content.

4. 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°C, 12 months
Reagent 2	Chromogenic Agent A	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, protected from light
Reagent 3	Chromogenic Agent B	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, protected from light
Reagent 4	Enzyme Reagent A	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protected from light
Reagent 5	Enzyme Reagent B	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protected from light
Reagent 6	Precipitator	12 mL × 1 vial	24 mL × 1 vial	-20°C, 12 months
Reagent 7	Acid Solution	12 mL × 1 vial	24 mL × 1 vial	-20°C, 12 months
Reagent 8	Alkali Solution	6 mL × 1 vial	12 mL × 1 vial	-20°C, 12 months
Reagent 9	1 mmol/L Standard Solution	1.8 mL × 1 vial	1.8 mL × 1 vial	-20°C, 12 months, protected from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (545-555 nm, optimum wavelength: 550 nm), Incubator (37°C)

Reagents:

Absolute ethanol

Consumables:

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

6. 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. **Preparation of enzyme reagent A working solution:** Dissolve one vial of enzyme reagent A with 6 mL of buffer solution, mix thoroughly and keep the solution on ice during use. Store aliquots at -20°C for 7 days protected from light.
3. **Preparation of enzyme reagent B working solution:** Dissolve one vial of enzyme reagent B with 0.2 mL of double distilled water, mix thoroughly. Store aliquots at -20°C for 7 days protected from light.
4. **Preparation of chromogenic working solution:** Before testing, prepare sufficient chromogenic working solution according to the number of test wells. For example, prepare 510 µL of chromogenic working solution (mix 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 fresh and used within 1 hour protected from light.
5. **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 serial concentrations. The recommended dilution gradient is: 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0 mmol/L.

7. Standard dilution table

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

8. Sample preparation

Sample preparation:

Serum and plasma: Centrifuge with 10 kDa MWCO spin filter at 12,000×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 12,000×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 thoroughly and stand at 2-8°C for 1 hour.

2. Centrifuge at 15,000×g for 10 min to remove supernatant. Add 1500 µL of double distilled water, mix thoroughly and centrifuge at 15,000×g for 10 min.

3. Remove the supernatant, add 100 µL of acid solution, mix thoroughly 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 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.

9. Sample dilution recommendations

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

10. Key points of the assay

During sample pretreatment step 2, use the pipette tip to fully suspend the precipitate to ensure even distribution.

11. Operating steps

1. Standard wells: Add 20 µL of standard with different concentrations into the standard wells. Sample wells: 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.

12. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding 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:

Oxalate content (mmol/L) = (ΔA - b) ÷ a × f × 3*

2. Tissue sample:

Oxalate content (mmol/kg wet weight) = (ΔA - b) ÷ a ÷ (m/v) × f × 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

13. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision table

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.

Inter-assay Precision table

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

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 replicates for each concentration to obtain an average recovery rate of 98.7%.

Recovery table

Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.10	0.50	0.80

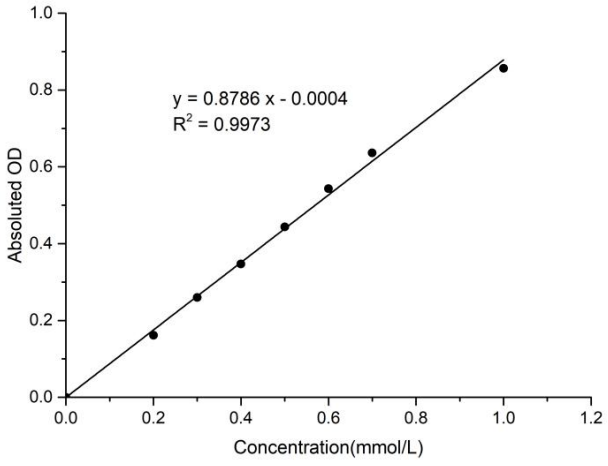
Parameters	Standard 1	Standard 2	Standard 3
Observed Conc. (mmol/L)	0.10	0.50	0.82
Recovery rate (%)	98	100	98

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.

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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	OD 1	OD 2	Average OD	Absolute OD
0	0.042	0.043	0.043	0.000
0.2	0.211	0.197	0.204	0.162
0.3	0.302	0.303	0.303	0.260

Concentration (mmol/L)	OD 1	OD 2	Average OD	Absolute OD
0.4	0.392	0.387	0.390	0.347
0.5	0.502	0.470	0.486	0.444
0.6	0.578	0.593	0.586	0.543
0.7	0.682	0.675	0.679	0.636
1.0	0.926	0.872	0.899	0.857

14. Appendix II Example Analysis

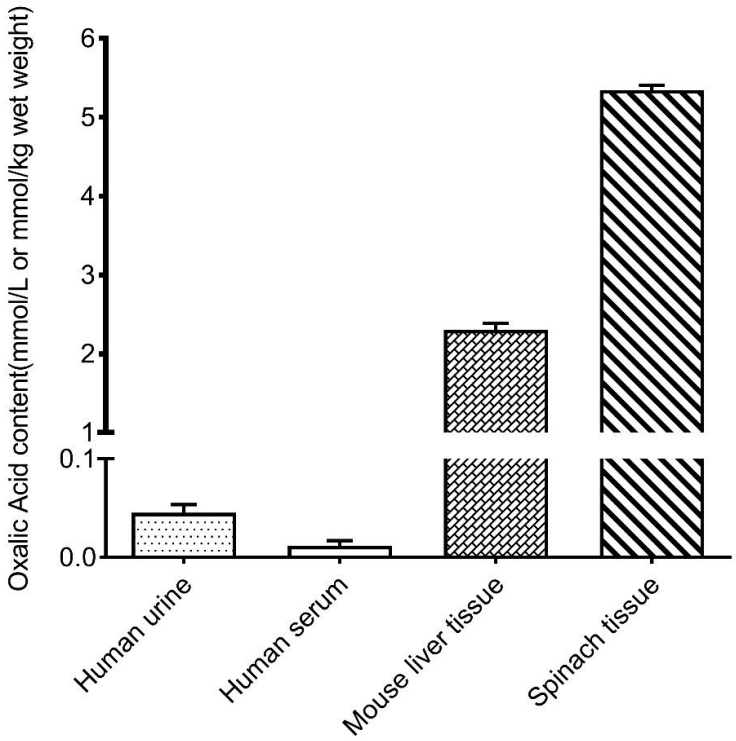
Example analysis:

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

The standard curve: y = 0.8786x - 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:

Oxalate content (mmol/L) = (0.055 - 0.043 + 0.0004) ÷ 0.8786 × 3 = 0.042 mmol/L

Detection of human urine, human serum, 10% mouse liver tissue homogenate and 10% spinach tissue homogenate according to the protocol:



15. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.