Technical Manual

Oxalate (Oxalic Acid) Colorimetric Assay Kit

- Catalogue Code: MAES0204
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.02-1 mmol/L

Sensitivity:

0.02 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be followed strictly, changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

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2. Background

Oxalic acid or oxalate is the metabolic decomposition product of the glyoxalate cycle in eukaryotes. The concentration of oxalate in human urine and other mammals can be used to evaluate renal function. High levels of oxalate may lead to the development of kidney stones, which are mainly composed of insoluble calcium oxalate.

3. Intended Use

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

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	12 mL × 1 vial	2-8˚C, 6 months
Working Solution	12 mL × 1 vial	2-8˚C, 6 months, avoid direct sunlight
Chromogenic Agent A	12 mL × 1 vial	2-8˚C, 6 months, avoid direct sunlight
Chromogenic Agent B	2 vials Lyophilized	2-8˚C 6 months
Enzyme Reagent A	1 vial Lyophilized	2-8˚C, 6 months
Enzyme Reagent B	12 mL × 1 vial	2-8˚C, 6 months
Regulator	2 mL × 1 vial	2-8˚C, 6 months
Clarificant	2 mL × 1 vial	2-8˚C, 6 months
Standard (1 mmol/L)	96 wells	No requirement
Microplate	2 pieces	
Plate Sealer	12 mL × 1 vial	2-8˚C, 6 months

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Materials required but not supplied

- Micropipettor
- 37˚C incubator
- Centrifuge
- Microplate Reader (545-555 nm, optimum wavelength: 550 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes

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.

7. Reagent Preparation

1. Bring all reagents to room temperature before use.

2. Preparation of **Chromogenic Agent B working solution**: dissolve a vial of Chromogenic Agent B powder with 5 mL of Buffer Solution fully and preserve it on ice for detection. The prepared solution should be used in 6 h.

3. Preparation of **Enzyme Reagent A working solution**: dissolve a vial of Enzyme Reagent A powder with 0.5 mL of double distilled water.

4. Preparation of **reaction working solution**: mix the Working Solution, Chromogenic Agent A and Enzyme Reagent A working solution at the ratio of 100:100:1. Prepare the fresh needed amount before use and prepared solution should be used in 1 h.

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8. Sample Preparation

1. **Serum sample:**
   Detect the sample directly.

2. **Urine sample:**
   Mix the sample with Enzyme Reagent B at a ratio of 1: 1. Stand at room temperature for 10 min for detection.

3. **Plant tissue sample:**
   Weigh the tissue accurately. Add double distilled water in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 10000 g for 10 min at 4˚C and collect the supernatant. Mix the supernatant with Enzyme Reagent B at a ratio of 1: 1. Stand at room temperature for 10 min for detection.

Dilution of Samples:

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.02-1 mmol/L).

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.

Sample Notes:

Predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

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9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 550 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1′	S9	S9′	S17	S17′	S25	S25′	S33	S33′
B	B	B	S2	S2′	S10	S10′	S18	S18′	S26	S26′	S34	S34′
C	C	C	S3	S3′	S11	S11′	S19	S19′	S27	S27′	S35	S35′
D	D	D	S4	S4′	S12	S12′	S20	S20′	S28	S28′	S36	S36′
E	E	E	S5	S5′	S13	S13′	S21	S21′	S29	S29′	S37	S37′
F	F	F	S6	S6′	S14	S14′	S22	S22′	S30	S30′	S38	S38′
G	G	G	S7	S7′	S15	S15′	S23	S23′	S31	S31′	S39	S39′
H	H	H	S8	S8′	S16	S16′	S24	S24′	S32	S32′	S40	S40′

Note: A-H, standard wells; S1-S40, sample wells; S1′-S40′,control wells.

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10. Operation Steps

Preparation of the Standard Curve:

Dilute 1 mmol/L standard solution 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 mmol/L.

The Measurement of Sample:

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 Chromogenic Agent B working solution into sample well and standard well. Add 80 μL of double distilled water into control well.

3. Incubate at 37°C 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 Regulator into each well.

7. Mix fully with microplate reader 5s and measure the OD value of each well at 550 nm with microplate reader.

Operation Table

	Standard Well	Sample Well	Dilution Factor
Standard of different concentrations (μL)	10		
Sample (μL)		10	10
Chromogenic Agent B working solution (μL)	80	80	
Double distilled water (μL)			80
Incubate at 37°C for 10 min.			
Reaction working solution (μL)	120	120	120
Stand at room temperature for 2 min.			
Regulator (μL)	20	20	20
Mix fully with microplate reader 5s and measure the OD value of each well at 550 nm with microplate reader.			

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12. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample has been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor. The standard curve is y= ax * b.

1. Urine sample:

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

2. Serum/Plasma sample:

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

3. Tissue sample:

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
x: The concentration of standard
a: The slope of the standard curve
b: The intercept of the standard curve
ΔA: $OD_{Sample} - OD_{Control}$
2*: Dilution factor of sample in sample pre-treatment 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

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13. Performance Characteristics

Detection Range	0.02-1 mmol/L
Sensitivity	0.02 mmol/L
Average recovery rate (%)	95
Average inter-assay CV (%)	4.7
Average intra-assay CV (%)	3

Analysis

For human urine, take 10 μL of human urine and complete 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:

$$\boldsymbol{Oxalate\ content}\left(\frac{\boldsymbol{mmol}}{\boldsymbol{L}}\right) = (\mathbf{0.352} - \mathbf{0.216} - \mathbf{0.0072}) \div \mathbf{0.4236} \times \mathbf{2}$$
$$= \mathbf{0.60}\ \boldsymbol{mmol/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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