PRODUCT INFORMATION & MANUAL

Oxalic Acid/Oxalate Assay Kit (Colorimetric)

NBP3-25894

For research use only.
Not for diagnostic or therapeutic procedures.

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Oxalic Acid/Oxalate Assay Kit
(Colorimetric)

Catalog No: NBP3-25894

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.02 mmol/L

Detection range: 0.02-1 mmol/L

Average intra-assay CV (%): 3.0

Average inter-assay CV (%): 4.7

Average recovery rate (%): 95

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

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.

▲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	Specification	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.			

▲Materials prepared by users

Instruments

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

Reagents:

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ 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.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 4 working solution:

   Dissolve a vial of reagent 4 powder with 5 mL of reagent 1 fully and preserve it on ice for detection. The prepared solution can be stored at 2~8℃ for 2 days.
3. Preparation of reagent 5 working solution:

   Dissolve a vial of reagent 5 powder with 0.25 mL of double distilled water. The prepared solution can be stored at 2~8℃ for 2 days.
4. Preparation of reaction working solution:

   Mix the reagent 2, reagent 3 and reagent 5 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.

▲ Sample preparation

1. Serum (plasma): Detect the sample directly.
2. Urine: Mix the sample with reagent 6 at a ratio of 1: 1. Stand at room temperature for 10 min for detection.
3. Plant tissue: 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℃ and collect the supernatant. Mix the supernatant with reagent 6 at a ratio of 1: 1. Stand at room temperature for 10 min for detection.

▲Dilution of sample

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.

Assay protocol

▲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.

▲Detailed operating steps

1. The preparation of standard curve

Dilute1 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, 1.0 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L Standard (μL)	Double distilled water (μL)
A	0	0	200
B	0.2	40	160
C	0.4	80	120
D	0.5	100	100
E	0.6	120	80
F	0.7	140	60
G	0.8	160	40
H	1.0	200	0

2. The measurement of samples

(1) Sample well: Add 10 μL of sample to corresponding well.

Control well: Add 10 μL of sample to corresponding well.

Standard welll: Add 10 μL of standard with different concentrations to corresponding well.

(2) Add 80 μL of reagent 4 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 reagent 7 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.

▲Summary operation table

	Standard well	Sample well	Control well
Standard of different concentrations (μL)	10		
Sample (μL)		10	10
Reagent 4 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.			
Reagent 7 (μL)	20	20	20
Mix fully and measure the OD value at 550 nm.			

▲Calculation

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. The standard curve is: y= ax + b.

1. Urine samples:

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

2. Serum/plasma samples:

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

3. Plant tissue samples:

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

Note:

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 standard curve.

b: The intercept of standard curve.

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 Data

▲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=0.60mmol/L

Appendix II References

1. Buttery J E , Ludvigsen N , Braiotta E A , et al. Determination of urinary oxalate with commercially available oxalate oxidase.[J]. Clinical Chemistry, 1983(4): 700-2.
2. Berckmans R J , Boer P . An inexpensive method for sensitive enzymatic determination of oxalate in urine and plasma.[J]. Clinical Chemistry, 1988(7): 1451-5.