

ab196990

Oxalate Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Oxalate in a variety of samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

INTRODUCTION

- 1. OVERVIEW 2
- 2. ASSAY SUMMARY 3

GENERAL INFORMATION

- 3. PRECAUTIONS 4
- 4. STORAGE AND STABILITY 4
- 5. MATERIALS SUPPLIED 5
- 6. MATERIALS REQUIRED, NOT SUPPLIED 5
- 7. LIMITATIONS 6
- 8. TECHNICAL HINTS 7

ASSAY PREPARATION

- 9. REAGENT PREPARATION 8
- 10. STANDARD PREPARATIONS 9
- 11. SAMPLE PREPARATION 10

ASSAY PROCEDURE and DETECTION

- 12. ASSAY PROCEDURE and DETECTION 12

DATA ANALYSIS

- 13. CALCULATIONS 14
- 14. TYPICAL DATA 16

RESOURCES

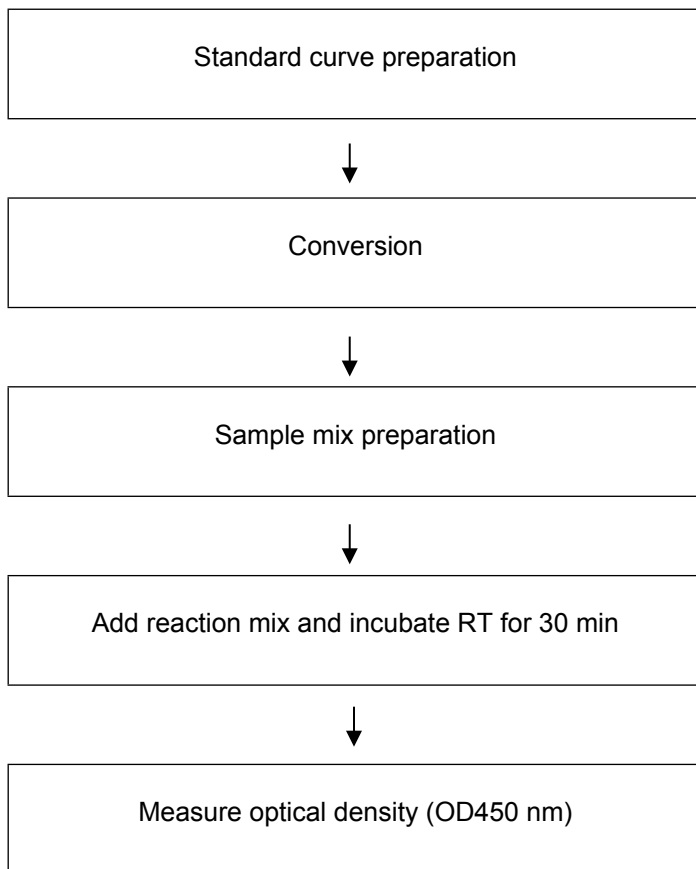
- 15. QUICK ASSAY PROCEDURE 17
- 16. TROUBLESHOOTING 18
- 17. FAQ 20
- 18. INTERFERENCES 21
- 19. NOTES 22

1. OVERVIEW

Oxalate Assay kit (Colorimetric) (ab196990) is an easy-to-use, sensitive and high throughput adaptable kit. In this assay, oxalate reacts with oxalate converter and oxalate enzyme mix to form an intermediate, which in turn reacts with a highly specific developer solution/probe to generate color at OD=450 nm. The assay kit can detect oxalate levels lower than 20 μM .

Oxalate ($\text{C}_2\text{O}_4^{2-}$), in the form of Oxalic acid is present in many foods and beverages (e.g. spinach, tea etc.). It accumulates in many plant tissues and play role in regulating pH, osmosis and calcium storage. In animals, oxalate is either absorbed from dietary intake or produced from glycolate metabolism in liver. Under normal conditions, the daily oxalate load can be excreted by kidney. However, hereditary defects can cause an increased level of oxalate, which leads to hyperoxaluria and results in the formation of kidney stones. Therefore, measurement of oxalate level is useful for the prevention, diagnosis and monitoring of kidney stones.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Briefly centrifuge small vials prior to opening. All kit components are supplied as ready to be used. Keep on ice while in use.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) | Storage Condition (After Preparation) |
|--|--------|--|---------------------------------------|
| Assay Buffer XXI/Oxalate Assay Buffer | 25 mL | -20°C | 4°C or -20°C |
| Buffer II/Oxalate Development Buffer | 15 mL | -20°C | 4°C or -20°C |
| Oxalate Converter | 200 µL | -20°C | -20°C |
| Enzyme Mix IX/Oxalate Enzyme Mix (lyophilized) | 1 vial | -20°C | -20°C |
| Developer Solution III/Oxalate Probe (lyophilized) | 1 vial | -20°C | -20°C |
| Oxalate Standard/Oxalate Standard (lyophilized) | 1 vial | -20°C | -20°C |

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear, flat bottom plates for colorimetric assay
- Heat block or water bath
- Orbital shaker
- Vortex
- Activated charcoal (>80 mesh)
- Mortar and pestle
- 10 M Hydrochloric acid (for urine samples only)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.
 - 9.1 **Oxalate Buffer XXI/Oxalate Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.
 - 9.2 **Buffer II/Oxalate Development Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.
 - 9.3 **Oxalate Converter:**

Ready to use as supplied. Aliquot converter so that you have enough volume to perform the desired number of assays. Store at -20°C and use within two months. Keep on ice while in use.
 - 9.4 **Enzyme Mix IX/Oxalate Enzyme Mix:**

Reconstitute with 220 μL ddH₂O. Pipette up and down to dissolve completely. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C. Use within two months. Keep on ice while in use.
 - 9.5 **Developer Solution III/Oxalate Probe:**

Reconstitute with 220 μL ddH₂O. Aliquot developer solution/probe so that you have enough volume to perform the desired number of assays. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.
 - 9.6 **Oxalate Standard:**

Reconstitute with 100 μL ddH₂O to generate 100 mM (100 nmol/ μL) Oxalate Standard solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare 1 mM Oxalate Standard Solution by adding 10 μL 100 mM Oxalate Standard into 990 μL of ddH₂O.

10.2 Using 1 mM Oxalate Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| Standard # | Volume of Standard (μL) | Assay Buffer (μL) | Final volume standard in well (μL) | End Conc. Oxalate in well |
|------------|--------------------------------------|--------------------------------|---|---------------------------|
| 1 | 0 | 150 | 50 μL | 0 nmol/well |
| 2 | 6 | 144 | 50 μL | 2 nmol/well |
| 3 | 12 | 138 | 50 μL | 4 nmol/well |
| 4 | 18 | 132 | 50 μL | 6 nmol/well |
| 5 | 24 | 126 | 50 μL | 8 nmol/well |
| 6 | 30 | 120 | 50 μL | 10 nmol/well |

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 μL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Plant Tissue or Fruit samples:

11.2.1 Harvest the amount of plant tissue or fruit necessary for each assay (initial recommendation = 15 mg). Grind in pre-cooled mortar with pestle to break the cell wall until a paste is formed from grinding (for the most efficient extraction, quickly freeze under liquid nitrogen and grind in a pre-cooled mortar using a pestle. Add 150 μL of ice-cold Assay Buffer XXI/Oxalate Assay Buffer to tissue/fruit paste/powder and homogenize. Incubate the homogenate for 10 minutes on ice and centrifuge at 10,000 $\times g$ for 5 minutes

11.2 Urine sample:

11.3.1 For urine samples, we recommend collecting samples 24 hours prior to testing. Collect sample (10 mL) in a bottle containing 10 mL of 10 M hydrochloric acid.

11.3.4 Centrifuge at 1,000 $\times g$ for 10 minutes and either use the supernatant immediately for analysis or save at -20°C for future experiments.

11.3.4 To reduce background from urine samples, we recommend purifying the urine samples further by mixing 1 mL of urine

with activated charcoal (25 mg) [not provided] for 5 minutes at room temperature.

- 11.3.4 Centrifuge at 10,000 x *g* for 5 minutes and use 10 μ L of the supernatant for the assay.

11.3 Other liquid samples (serum, plasma, other fluids):

Other liquid samples (e.g. serum) can be tested directly by adding sample to the microplate wells.

NOTE: We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of oxalate in the test samples, we recommend spiking samples with a known amount of Standard (4 nmol).

12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:

- Standard wells = 50 μ L standard dilutions.
- Sample wells = 1 – 50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer XXI/Oxalate Assay Buffer).
- Spiked Sample= 1 – 50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer XXI/Oxalate Assay Buffer) + 4 μ L 1 mM Oxalate standard solution (Section 10.1). **NOTE:** for samples that can interfere such as urine samples.

12.2 Conversion:

- Add 2 μ L of Oxalate Converter to each standard, sample and Spiked Sample wells.
- Mix and incubate at 37°C for 1 hour.

12.3 Reaction Mix:

Prepare 50 μ L of Reaction Mix for each reaction

| Component | Colorimetric Reaction Mix (μ L) |
|--------------------------------------|--------------------------------------|
| Buffer II/Oxalate Development Buffer | 46 |
| Enzyme Mix IX/Oxalate Enzyme Mix | 2 |
| Developer Solution III/Oxalate Probe | 2 |

Mix enough reagents for the number of assays (samples, standards and Spiked Samples) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X μ L component x (Number samples + Standards + Spiked Samples + 1).

- 12.4 Add 50 μ L of Reaction Mix into each standard, sample and Spiked Sample wells.
- 12.5 Mix and incubate at 37°C for 60 minutes protected from light.
- 12.6 Measure output on a colorimetric microplate reader at OD450 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of oxalate.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$x = \left(\frac{\text{Corrected absorbance} - (y - \text{intercept})}{\text{Slope}} \right)$$

13.6 Apply the corrected sample reading to Standard Curve to get B nmol of Oxalate amount in the sample wells.

13.7 Concentration of oxalate (nmol/mL = μM) in the test samples is calculated as:

$$\text{Oxalate Concentration (C)} = \frac{B}{V} \times D$$

Where:

B = Amount of Oxalate from Standard Curve (nmol)

V = Sample volume added into the reaction well (mL)

D = Sample Dilution factor

For samples with interference calculate the Oxalate amount in nmol by subtracting the sample reading from the Spiked Sample reading using the following equation:

$$\text{Oxalate} = \left(\frac{(\text{OD}_{\text{sample corrected}})}{(\text{OD}_{\text{spiked sample corrected}}) - (\text{OD}_{\text{sample corrected}})} \right) * 4 \text{ nmol}$$

Oxalic acid molecular weight: 90 g/mol.

Oxalate in samples can also be expressed in nmol/mg of sample.

14. TYPICAL DATA

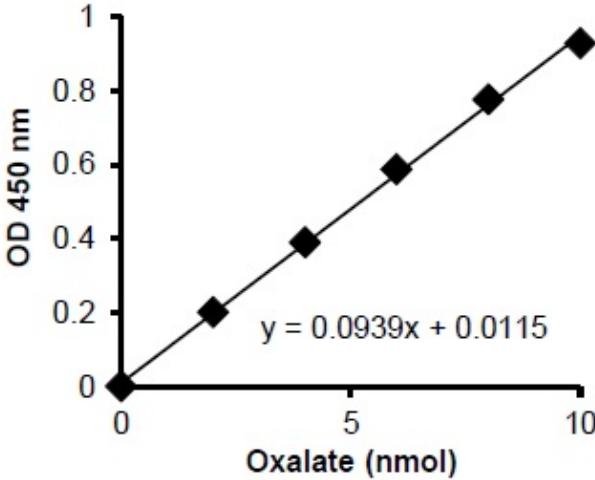


Figure 1: Typical Oxalate Standard calibration curve following assay procedure.

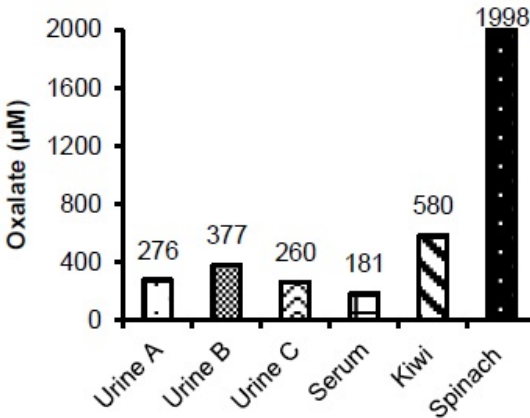


Figure 2: Oxalate concentration in normal human urine (3 donors, 10 µL sample), normal human serum (10 µL), kiwi lysate (10 µL) and spinach lysate (10 µL).

15. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare standard, developer solution/probe, converter, enzyme mix, development and assay buffers; (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 μ L), samples (50 μ L) and background wells (50 μ L).
- Add 2 μ L of Oxalate Converter to each standard, sample and background sample well.
- Mix and incubate plate at 37°C 1 hour.
- Prepare Oxalate Reaction Mix (Number samples + standards + background sample control + 1).

| Component | Colorimetric Reaction Mix (μ L) |
|--------------------------------------|--------------------------------------|
| Buffer II/Oxalate Development Buffer | 46 |
| Enzyme Mix IX/Oxalate Enzyme Mix | 2 |
| Developer Solution III/Oxalate Probe | 2 |

- Add 50 μ L of Oxalate Reaction Mix to the standard and sample wells.
- Incubate plate at 37°C 60 minutes protected from light.
- Measure plate at OD 450 nm.

16. TROUBLESHOOTING

| Problem | Cause | Solution |
|--|--|---|
| Assay not working | Use of ice-cold buffer | Buffers must be at room temperature |
| | Plate read at incorrect wavelength | Check the wavelength and filter settings of instrument |
| | Use of a different 96-well plate | Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate |
| Sample with erratic readings | Samples not deproteinized (if indicated on protocol) | Use PCA precipitation protocol for deproteinization |
| | Cells/tissue samples not homogenized completely | Use Dounce homogenizer, increase number of strokes |
| | Samples used after multiple free/ thaw cycles | Aliquot and freeze samples if needed to use multiple times |
| | Use of old or inappropriately stored samples | Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use |
| | Presence of interfering substance in the sample | Check protocol for interfering substances; deproteinize samples |
| Lower/ Higher readings in samples and Standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Allowing reagents to sit for extended times on ice | Always thaw and prepare fresh reaction mix before use |
| | Incorrect incubation times or temperatures | Verify correct incubation times and temperatures in protocol |

RESOURCES

| Problem | Cause | Solution |
|--|---|---|
| Standard readings do not follow a linear pattern | Pipetting errors in standard or reaction mix | Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the tubes |
| | Standard stock is at incorrect concentration | Always refer to dilutions described in the protocol |
| Unanticipated results | Measured at incorrect wavelength | Check equipment and filter setting |
| | Samples contain interfering substances | Troubleshoot if it interferes with the kit |
| | Sample readings above/ below the linear range | Concentrate/ Dilute sample so it is within the linear range |

17. FAQ

Can this kit be used with plant tissue?

Our Oxalic acid assay can work with a variety of samples as long as there is oxalic acid in the range of the standard. curve. This kit can detect Oxalate levels lower than 20 μM . Please follow the protocol mention on the datasheet for plant tissues.

How does adding oxalate standard into the sample help?

Spiking a parallel control sample with a known amount of oxalate standard helps to account for any interference from endogenous sources in the sample. 4 μL of 1 mM Oxalate is 4 nmoles of oxalate and this amount should be recovered when the OD is read.

18. INTERFERENCES

19. NOTES

Technical Support

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