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Product Information

Oxalate Assay Kit

Catalog Number **MAK315**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Oxalate or Oxalic Acid is a metabolic breakdown product of the Kreb's Cycle in eukaryotes, and the glyoxylate cycle in other microorganisms. It can be found in the urine of humans and other mammals. Oxalate concentration can be used as a measure of kidney function where a high level of oxalate is an indicator for kidney stones, which are primarily made of the insoluble salt calcium oxalate. Measuring oxalate is more accurate than measuring calcium as a marker for kidney stones because calcium is excreted at high concentrations even in normal urine.

Simple, direct and high-throughput assays for measuring oxalate concentration find wide applications. This oxalate assay kit uses a single Working Reagent that combines the oxalate oxidase reaction and color reaction in one step. The change in color intensity of the reaction product at 595 nm is directly proportional to oxalate in the sample.

Key Features
Sensitive and accurate – Use samples as small as 10 µL. Linear detection range in 96 well plate for 10 minute incubation: 20–1,500 µM oxalate.

Fast and convenient – Sample pretreatment is faster and easier than using activated carbon in competitor's assay kits.

High-throughput adaptable – The procedure involves addition of a single working reagent and incubation for 10 minutes at room temperature. Can be automated for processing thousands of samples per day.

Components
The kit is sufficient for 100 assays in 96 well plates.

Reagent A Catalog Number MAK307A	100 µL
Reagent B Catalog Number MAK307B	18 mL
Standard, 500 µM Oxalate Catalog Number MAK307C	1 mL
HRP Enzyme Catalog Number MAK307D	120 µL
OX Enzyme Catalog Number MAK307E	120 µL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Storage/Stability
The kit is shipped on ice. Store all components at –20 °C upon receiving.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of reagents and samples.

Samples can be analyzed immediately after collection or stored in aliquots at 4 °C or –20 °C for 7 days. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use the clear supernatant for the assay. Equilibrate all components to room temperature. During experiment, keep thawed Enzymes in a refrigerator or on ice.

1. Transfer 10 µL of each sample into three separate wells. Three wells will be needed per sample: Sample Blank, Sample, and Internal Standard.

2. Add 10 µL of ultrapure water to Sample Blank and Sample wells, and 10 µL of Standard to the Internal Standard well.

3. Quench (For urine samples only. Go to step 4 for non-urine samples). Mix 5 µL of Reagent A to 20 mL of water. Add 30 µL of the diluted Reagent A to each well, tap plate lightly on the sides, and incubate for 2 minutes at room temperature.

4. Working Reagent – For Sample Blank wells, prepare enough Blank Reagent for all blank wells by mixing, for each 96 well assay, 155 µL of Reagent B and 1 µL of HRP Enzyme (i.e., No OX Enzyme).

   For Sample and Internal Standard wells, prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96 well assay, 155 µL of Reagent B, 1 µL of OX Enzyme, and 1 µL of HRP Enzyme.

   Note: Working Reagent and Blank Reagent are stable for 2 hours. It is recommended to prepare fresh reagents just prior to each assay run.

5. Add 150 µL of Blank Reagent to the Sample Blank wells, and 150 µL of Working Reagent to Sample and Internal Standard wells. Mix.

6. Incubate 10 minutes at room temperature, and then read the optical density at 595 nm (550–610 nm).

Procedure Using Cuvette
The following procedure is for use in 1 mL cuvettes; adjust volumes up or down in the same ratios for different cuvette sizes.

1. Transfer 25 µL of each sample into three separate cuvettes. Three cuvettes will be needed per sample: Sample Blank, Sample, and Internal Standard.
2. Add 25 µL of water to Sample Blank and Sample cuvettes, and 25 µL of Standard to the Internal Standard cuvette.
3. Quench (For urine samples only. Go to step 4 for non-urine samples). Mix 5 µL of Reagent A to 20 mL of water. Add 75 µL of the diluted Reagent A to each cuvette, mix lightly, and incubate for 2 minutes at room temperature.
4. Working Reagent – For Sample Blank cuvettes, prepare enough Blank Reagent for all blank cuvettes by mixing, per cuvette, 900 µL of Reagent B and 6 µL of HRP Enzyme (i.e., No OX Enzyme).

   For Sample and Internal Standard cuvettes, prepare enough Working Reagent for all cuvettes by mixing, per cuvette, 900 µL of Reagent B, 6 µL of OX Enzyme, and 6 µL of HRP Enzyme.
5. Add 875 µL of Blank Reagent to the Sample Blank cuvettes, and 875 µL of Working Reagent to Internal Standard and Sample cuvettes. Mix.
6. Incubate 10 minutes at room temperature, and then read the optical density at 595 nm (550–610 nm).

Results
Calculation
Oxalate concentration of a Sample is calculated as

$$[\text{Oxalate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 500 \times n \quad (\mu M)$$

Where:
OD_{SAMPLE}, $OD_{STANDARD}$, and OD_{BLANK} are the optical density values of the Sample, Internal Standard, and Sample Blank wells, respectively.
500 µM is the effective concentration of the Internal Standard, and n is the dilution factor.

Note: If the Sample oxalate concentration is higher than 1,000 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

References

1. Zuo, G. et al., A novel urinary oxalate determination method via catalase model compound with oxalate oxidase. Analytical Methods, **2010**(2), 254-258 (2009).

2. Hagen, L. et al., Plasma and Urinary Oxylate and Glycolate in Healthy Subjects. Clin. Chem., **39**(1), 134-138 (1993).

3. Costello, J., and Landwehr, D.M., Determination of Oxalate Concentration in Blood. Clin. Chem., **34**(8), 1540-1544 (1988).

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Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range