Technical Bulletin

Oxalate Assay Kit

Catalogue number MAK315

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.[1] 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.[1]
Simple, direct and high-throughput assays for measuring oxalate concentration find wide applications. The 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.

Sensitive and accurate – Use samples as small as 10µL. 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 colorimetric assays in 96-well plates.

- Reagent A 100 µL
  Catalogue Number MAK315A
- Reagent B 18 mL
  Catalogue Number MAK315B
- Standard, 500 µM Oxalate 1 mL
  Catalogue Number MAK315C
- HRP Enzyme 120 µL
  Catalogue Number MAK315D
- OX Enzyme 120 µL
  Catalogue Number MAK315E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor).
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plate. Cell culture or tissue culture treated plates are not recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on ice. Store all components at -20 °C upon receiving.

Preparation Instructions

Briefly centrifuge small vials prior to opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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.

Procedure for 96-well Plate

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 (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.

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:

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

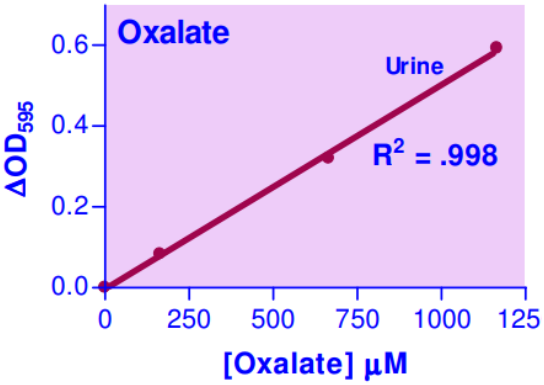
Where:

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.

Figure 1.
Typical Oxalate Standard Curve



References

1. Bargagli M, *et al*., Dietary Oxalate Intake and Kidney Outcomes. *Nutrients*., **12(9)**:2673. (2020).

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