EnzyChrom™ Oxalate Assay Kit (EOXA-100)

Quantitative Colorimetric Oxalate Determination

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. BioAssay Systems' 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 as little as 10 µL samples. Linear detection range in 96-well plate for 10 minute incubation: 20 to 1500 µM oxalate.

Fast and convenient. Sample pre-treatment 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 min at room temperature. Can be automated for processing thousands of samples per day.

APPLICATIONS

Direct Assays: oxalate concentration in urine, animal, and plant tissue samples.

Drug Discovery/Pharmacology: effects of drugs on oxalate concentration, metabolism, and excretion.

KIT CONTENTS (100 tests in 96-well plates)

Reagent A:	100 µL	**HRP Enzyme:**	120 µL
Reagent B:	18 mL	**OX Enzyme:**	120 µL
Standard:	1 mL 500 µM Oxalate		

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE

Samples can be analyzed immediately after collection, or stored in aliquots at 4°C or –20°C for 7 days. For longer term storage, acidify samples by adding 20 µL 6N HCl per 1 mL of Sample and store at -80°C. Prior to assaying, neutralize Sample with 20 µL 6N NaOH. Correct for dilution factor by multiplying by *n* = 1.04. 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 dH_2O to Sample Blank and Sample wells, and 10 µL of Standard to the Internal Standard well.
3. *Quench (For urine only. Move on to step 4 if your sample is not urine*). Mix 5 µL of Reagent A to 20 mL of dH_2O. 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 Reagent B and 1 µL 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 Reagent B, 1 µL OX Enzyme, and 1 µL HRP Enzyme.

   Note: Working Reagent and Blank Reagent are stable for 2 hours, we recommend making fresh reagents for each assay run.

   Add 150 µL Blank Reagent to the Sample Blank wells, and 150 µL Working Reagent to Sample and Internal Standard wells. Mix.
5. Incubate 10 min 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; you may scale the volumes up or down in the same ratios to adjust to your cuvette size.

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 dH_2O to Sample Blank and Sample cuvettes, and 25 µL of Standard to the Internal Standard cuvette.
3. *Quench (For urine only. Move on to step 4 if your sample is not urine*). Mix 5 µL of Reagent A to 20 mL of dH_2O. 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 Reagent B and 6 µL 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 Reagent B, 6 µL OX Enzyme, and 6 µL HRP Enzyme.

   Add 875 µL Blank Reagent to the Sample Blank cuvettes, and 875 µL Working Reagent to Internal Standard and Sample cuvettes. Mix.
5. Incubate 10 min at room temperature, and then read the optical density at 595 nm (550 – 610 nm).

CALCULATION

Oxalate concentration of a Sample is calculated as

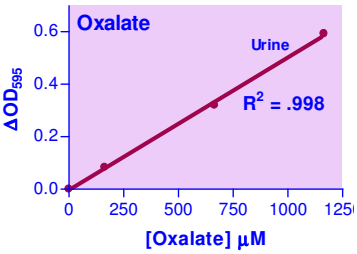
$$[\text{Oxalate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 500 \times n \quad (\mu M)$$

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 1000 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate or cuvette reader.



96-well colorimetric assay

Sample	[Oxalate]
Human Urine	212 ± 14 µM
Rat Serum	44 ± 13 µM
Human Serum	45 ± 6 µM
Bean Sprout*	0.303 ± 0.02 nmoles/mg
(n = 8) Spinach*	30.4 ± 1.2 nmoles/mg

**Solid samples are homogenized in phosphate buffered saline. Use approximately 100-200 mg per mL.*

Samples Tested (Not Reference Values)

LITERATURE

1. Zuo G, Jiang X et al. (2009). A novel urinary oxalate determination method via catalase model compound with oxalate oxidase. Analytical methods 2010(2):254-258
2. Hagen L, Walker VR, Sutton RAL. (1993). Plasma and Urinary Oxylate and Glycolate in Healthy Subjects. Clin Chem. 39(1):134-138
3. Costello J, Landwehr DM. (1988). Determination of Oxalate Concentration in Blood. Clin Chem. 34(8):1540-1544