Oxalate Assay Kit (Colorimetric)

Oxalate Assay Kit (Colorimetric) is a detection kit for the quantification of Oxalate in urine, animal and plant tissue samples.

Catalog number: ARG82186

Package: 100 tests

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INTRODUCTION

Oxalate (IUPAC: ethanedioate) is a compound found in some foods, which when consumed exits the body through the urine. Excess consumption has been linked to gout and kidney stones. Many metal ions form insoluble precipitates with oxalate, a prominent example being calcium oxalate, the primary constituent of the most common kind of kidney stones. Several plant foods such as the root and/or leaves of spinach, rhubarb, and buckwheat are high in oxalic acid and can contribute to the formation of kidney stones in some individuals. Chemically, oxalate is a dianion with the formula $C_2O_4^{2-}$, also written $(COO)_2^{2-}$. Either name is often used for derivatives, such as salts of oxalic acid, for example sodium oxalate $Na_2C_2O_4$, or dimethyl oxalate ($(CH_3)_2C_2O_4$). Oxalate also forms coordination compounds where it is sometimes abbreviated as ox. [Provide by Wikipedia: Oxalate]

PRINCIPLE OF THE ASSAY

This Oxalate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of oxalate in urine, animal and plant tissue. This assay 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 O.D. 595 nm is directly proportional to oxalate in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Reagent A	100 µL	-20°C
Reagent B	18 mL	-20°C
HRP Enzyme	120 µL	-20°C
OX Enzyme	120 µL	-20°C
Standard (500 µM Oxalate)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 595 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue lysate: homogenized in PBS followed by filtration or centrifugation (E.g., 5 minutes at 10,000 x g). Use approximately 100-200 mg per mL.

Urine: assayed directly. If particulates are present, centrifuge sample (5 minutes at 2,000 x g) and use the clear supernatant for the assay.

Note:

- All samples can be stored at 4 to -20°C for one week. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Working Reagent:** for each well, mixing 155 µL of Reagent B, 1 µL of OX Enzyme and 1 µL of HRP Enzyme. Fresh reconstitution is recommended.
- **Blank Working Reagent:** for each well, mixing 155 µL of Reagent B and 1 µL of HRP Enzyme (no OX enzyme). Fresh reconstitution is recommended.

Note: Working Reagent and Blank Working Reagent are stable for 2 hours.

ASSAY PROCEDURE

Equilibrate all components to room temperature. During experiment, keep thawed Enzymes in a refrigerator or on ice. Briefly centrifuge tubes before use.

	Internal Standard well	Sample well	Sample Blank well
Standards	10 µL		
Each Sample	10 µL	10 µL	10 µL
Distilled water		10 µL	10 µL
Quench (for urine sample only. Move on to next step if your sample is not urine). Mix **5 µL** of **Reagent A** and **20 mL** of **distilled water**. Add **30 µL** of the **diluted Reagent A** to each well. Tap plate lightly and incubate for **2 minutes** at **room temperature**.			
Working Reagent	150 µL	150 µL	
Blank Working Reagent			150 µL
Tap plate to mix immediately. Incubate for **10 minutes** at **room temperature**.			
Read the absorbance at O.D. 595 nm. (550-610 nm)			

CALCULATION OF RESULTS

1. Oxalate concentration of a sample is calculated as:

   Oxalate (µM) = $[(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Sample})] \times 500 \times n$

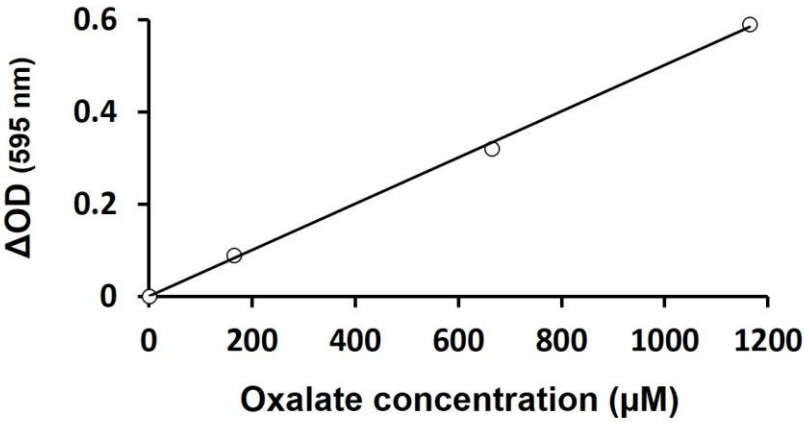
   Note:

   - OD_{Sample}, OD_{Blank} and $OD_{Standard}$: the O.D. 595 nm values of the Sample, Sample blank and Internal Standard.
   - 500 µM is the effective concentration of the Internal Standard
   - n: the sample dilution factor.

2. If the Sample oxalate concentration is higher than 1000 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Oxalate Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

20 µM