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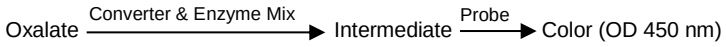
Oxalate (Oxalic Acid) Colorimetric Assay Kit

02/14

(Catalog # K663-100; 100 assays; Store at -20°C)

I. Introduction:

Oxalate ($C_2O_4^{2-}$), 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. BioVision's Oxalate Assay kit is an easy-to-use, sensitive and high throughput adaptable kit. In this assay, Oxalate reacts with Oxalate Converter & Oxalate Enzyme Mix to form an intermediate, which in turn reacts with a highly specific probe to generate color at 450 nm. The assay kit can detect Oxalate levels lower than 20 µM.



II. Application:

- Measurement of Oxalate level in various samples.
- Analysis of kidney and liver functions.

III. Sample Type:

- Animal samples: Urine, serum, plasma, kidney, liver etc.
- Plant samples: Vegetable leafs and fruits.

IV. Kit Contents:

Components	K663-100	Cap Code	Part Number
Oxalate Assay Buffer	25 ml	WM	K663-100-1
Oxalate Development Buffer	15 ml	NM	K663-100-2
Oxalate Converter	0.2 ml	Purple	K663-100-3
Oxalate Enzyme Mix (lyophilized)	1 vial	Green	K663-100-4
Oxalate Probe (lyophilized)	1 vial	Red	K663-100-5
Oxalate Standard (lyophilized)	1 vial	Yellow	K663-100-6

V. User Supplied Reagents and Equipments:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Activated charcoal (> 80 mesh)
- Mortar and pestle

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Read the entire protocol before performing the assay.

- **Oxalate Assay Buffer** and **Oxalate Development Buffer**: Warm buffers to room temperature before use. Store at 4°C or -20°C.
- **Oxalate Converter:** Aliquot and store at -20°C. Avoid repeated freeze-thaw. Use within two months.
- **Oxalate Enzyme Mix**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.
- **Oxalate Probe**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for two months at -20°C.
- **Oxalate Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) Oxalate Standard solution. Keep on ice while in use. Store at -20°C. Use within two months.

VII. Oxalate Assay Protocol:

1. **Sample Preparation:** Liquid samples (*e.g.* serum) can be measured directly. For plant tissues or fruits (~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 Oxalate Assay Buffer to tissue/fruit paste/powder and homogenize. Incubate the homogenate for 10 min. on ice and centrifuge at 10,000 X g for 5 min. For animal tissue, homogenize the tissue directly and centrifuge at 10,000 X g for 5 min. Collect the supernatant. Add 1-50 µl of the supernatant into a 96-well plate and bring the volume to 50 µl with Oxalate Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   b. For urine samples, we recommend collecting samples 24 hrs prior to testing. Collect sample (10 ml) in a bottle containing 10 ml of 10 M hydrochloric acid. Centrifuge at 1,000 X g for 10 min. and either use the supernatant immediately for analysis or save at -20ºC for future experiments.

   c. 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 min. at room temperature. Centrifuge at 10,000 X g for 5 min. and use 10 µl of the supernatant for the assay.

d. *To correct for sample interference (e.g. urine sample), we recommend doing samples in pairs to estimate sample background by adding 4 µl of 1 mM Oxalate Standard to one of each sample pair as an internal standard (sample+4).

2. **Standard Curve Preparation:** Dilute Oxalate Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Oxalate Standard to 990 µl dH_2O, mix well. Add 0, 2, 4, 6, 8, and 10 µl of the 1 mM Oxalate Standard into a series of wells in a 96-well plate. Adjust the volume to 50 µl/well with Oxalate Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of Oxalate Standard.
3. **Conversion:** Add 2 µl of Oxalate Converter to each Standard and samples well. Mix and incubate at 37ºC for 1 hr.
4. **Reaction Mix:** Mix enough reagents for number of assays (standard and samples) to be performed. For each well, prepare 50 µl of Reaction Mix containing:

	Reaction Mix
Oxalate Development Buffer	46 µl
Oxalate Enzyme Mix	2 µl
Oxalate Probe	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and samples, mix well.

5. **Measurement:** Incubate the reaction at 37ºC for 60 min. and measure absorbance (450 nm).
6. **Calculations:** Subtract 0 Oxalate Standard reading from all readings. Plot the Oxalate Standard Curve. Apply the corrected sample reading to Standard Curve to get B nmol of Oxalate amount in the sample wells.

Sample Oxalate concentration (C) = B/VxD = nmol/ml = µM

Where: **B** is the amount of Oxalate in the sample well from Standard Curve (nmol).
V is the sample volume used in the reaction well (ml).
D is the sample Dilution Factor.

***Note:** For samples with interference, correct sample interference by subtracting the sample reading from spiked sample reading using the following equation:

$$\textbf{Oxalate amount in the sample well, B (nmol)} = \left(\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{sample +4(corrected)}})-(OD_{\text{sample(corrected)}})}\right) * 4 \text{ nmol}$$

Oxalic acid molecular weight: 90 g/mol. Oxalate in samples can also be expressed in nmol/mg of sample.

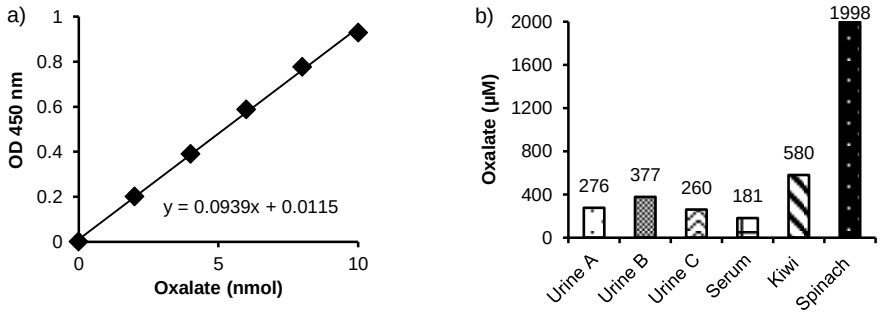


Figure: (a) Oxalate Standard Curve. (b) Oxalate concentration in normal human urine (3 donors, 10 µl sample), normal human serum (10 µl), Kiwi lysate (10 µl), and Spinach lysate (10 µl). Assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

Hydroxyproline Colorimetric Assay Kit (K555-100)
Lactate Dehydrogenese (LDH) Activity Assay Kit (K726-500)
Ascorbic Acid Colorimetric/Fluorometric Assay Kit (K661-100)
Ascorbic Acid Colorimetric Assay Kit II (FRASC) (K671-100)

FOR RESEARCH USE ONLY! Not to be used on humans.