



Oxalate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1246

(Version 1.1A)

Detection and Quantification of Oxalate Content in Urine, Serum,
Plasma and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Oxalate (or oxalic acid) is an organic acid produced as a metabolic byproduct in living organisms. In eukaryotes, it originates from the Krebs cycle, while in other microorganisms it is derived from the glyoxylate cycle. It can be detected in the urine of humans and other mammals. Urinary oxalate concentration serves as a crucial indicator for assessing kidney stone risk, as elevated levels are a primary warning sign for the formation of calcium oxalate stones. Measuring oxalate is a more accurate marker for kidney stones than measuring calcium, because calcium is excreted at high concentrations even in normal urine, which can easily mask pathological changes.

Oxalate Colorimetric Microplate Assay Kit provides a convenient tool for sensitive and specific detection of Oxalate in a variety of samples. The kit is based on linked enzymatic reaction catalyzed oxidation of Oxalate, which generates H_2O_2 and reacts with dye reagent to form a colored product. The color intensity increase at 550nm is proportional to the amount of Oxalate in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Quench	Powder x 1	-20 °C
Quench Buffer	5 ml x 1	4 °C
Reaction Buffer A	8 ml x 1	4 °C
Reaction Buffer B	10 ml x 1	4 °C
Enzyme Diluent	1 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C
Standard	Powder x 1	4 °C
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Note: Avoid freeze-thaw cycles

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 0.5 ml distilled water to generate 30 mmol/L of standard stock solution, store at 4 °C for 2-4 weeks or -20°C for 1-3 months after reconstitution. Then dilute to 3 mmol/L standard top solution by adding 50 µl stock solution into 450 µl distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.188/0.094/0.047 mmol/L.

Quench working solution: Prepare for urine only. Briefly centrifuge the vial of Quench prior to opening. Dissolve in 0.1 ml Quench buffer to generate Quench stock solution (100X). Keep in dark and store at 4 °C for 1 week or -20 °C for 2-3 months. Dilute to Quench working solution (1X) by adding 10 µl stock solution into 990 µl Quench Buffer. Prepare working solution fresh for immediate use.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Enzyme Diluent to dissolve before use, mix, store at 4°C for 3-4 days or -80°C for 3-4 months after reconstitution.

Dye Reagent: Briefly centrifuge prior to opening. Add 2 ml Reaction Buffer B to dissolve before use, mix, keep in dark and store at 4°C for 3-4 days or -20°C for 1-2 months after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For urine samples

Pipette 20 μ l urine sample into 30 μ l Quench working solution. Vortex gently and incubate for 2 minutes at room temperature.

2. For other liquid samples

Use directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer A	80 µl	80 µl	80 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Enzyme	10 µl	10 µl	10 µl
Mix and put in the oven at 37 °C for 20 minutes			
Reaction Buffer B	70 µl	70 µl	70 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix and put in the oven at 37 °C for 5 minutes, record absorbance measured at 550 nm			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n \text{ (mmol/L)}$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} \times \frac{V_{\text{Quench}} + V}{V} \text{ (}\mu\text{mol/ml)}$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard}: the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard}: the volume of standard in assay procedure, μl

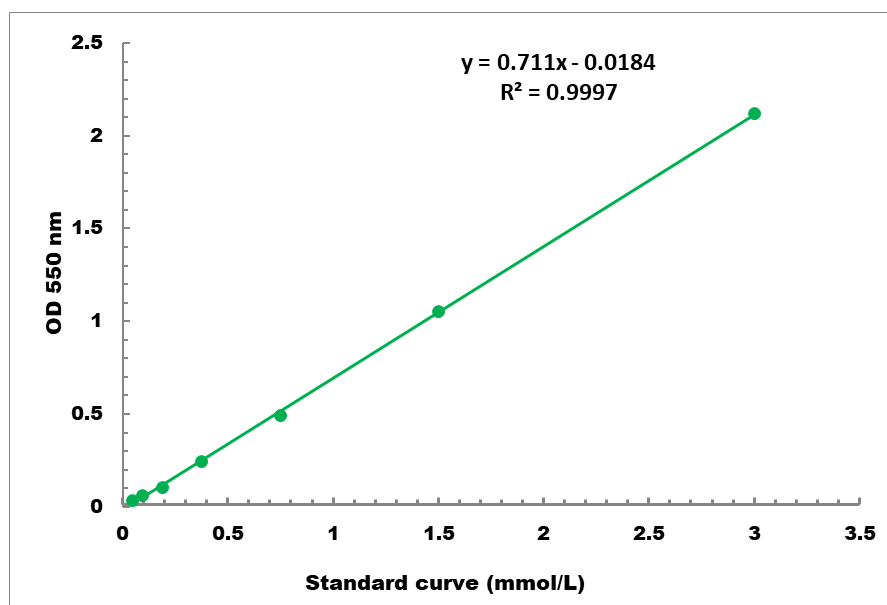
V_{Sample}: the volume of sample in assay procedure, μl

V_{Quench}: the volume of quench working solution, μl

V: the volume of sample in sample preparation, μl

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.03 mmol/L - 3 mmol/L