



Oxalate Oxidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1182

(Version 1.3C)

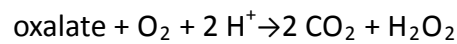
Detection and Quantification of Isocitrate Oxalate Oxidase (OXO)
Activity in Tissue extracts, Cell lysate, Cell culture media and Other
biological fluids Samples.

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

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I. INTRODUCTION

In enzymology, an oxalate oxidase (EC 1.2.3.4) is an enzyme that catalyzes the chemical reaction



The 3 substrates of this enzyme are oxalate, O_2 , and H^+ , whereas its two products are CO_2 and H_2O_2 .

This enzyme belongs to the family of oxidoreductases, specifically those acting on the aldehyde or oxo group of donor with oxygen as acceptor. The systematic name of this enzyme class is oxalate:oxygen oxidoreductase. Other names in common use include aëro-oxalo dehydrogenase, and oxalic acid oxidase. This enzyme participates in glyoxylate and dicarboxylate metabolism. It has 2 cofactors: FAD, and Manganese.

Oxalate Oxidase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining oxalate oxidase activity in various samples. Oxalate oxidase activity is determined by the product of H_2O_2 . The increase in absorbance at 555 nm is directly proportional to the enzyme activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	2 ml x 1	4 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Dye Reagent Diluent	16 ml x 1	4 °C
Standard (4 mmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 555 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. 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 4/2/1/0.5/0.25/0.125/0.0625 mmol/L.

Dye Reagent: Briefly centrifuge prior to opening. Add 1 ml Dye Reagent Diluent to dissolve, then transfer all reagent into Dye Reagent Diluent bottle, mix. Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 2 months.

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

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For other biological fluids samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Sample	20µl	--	--
Standard	--	20µl	--
Distilled water	--	--	20µl
Dye Reagent	160 µl	160 µl	160 µl
Mix.			
Substrate	20 µl	20 µl	20 µl
Mix, incubate at 30°C for 10 minutes, record absorbance measured at 555 nm.			

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: One Unit of OXO activity is defined as the enzyme produces 1 $\mu\text{mol H}_2\text{O}_2$ per min.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

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

2.1 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

2.4 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

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

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

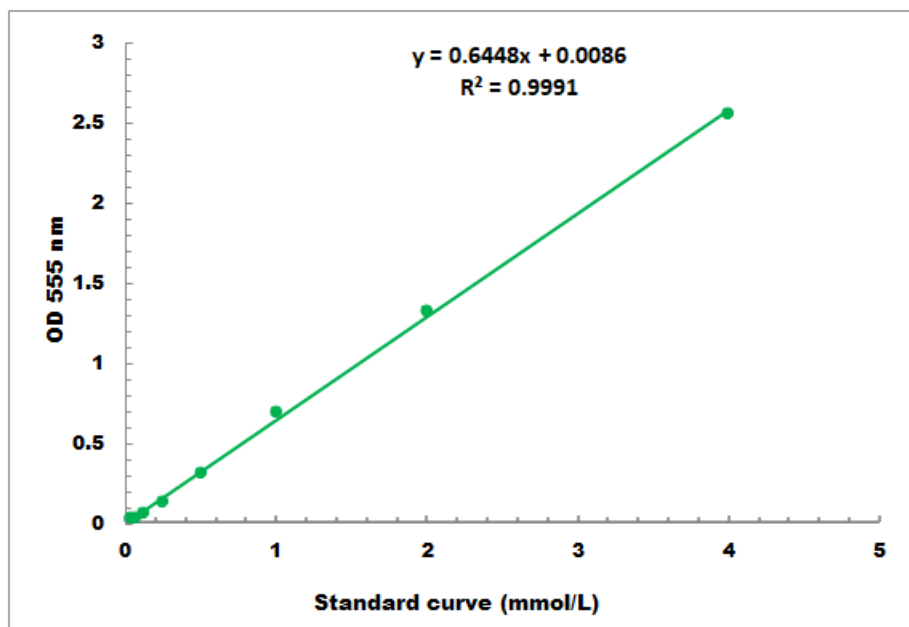
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.04 mmol/L - 4 mmol/L