



Oxalate Oxidase Microplate Assay Kit

User Manual

Catalog # CAK1182

(Version 1.2C)

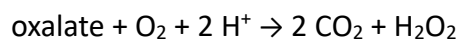
Detection and Quantification of Isocitrate Oxalate Oxidase (OXO)
Activity in Tissue extracts, Cell lysate, Cell culture media and Other
biological fluids Samples.

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, O₂, and H⁺, whereas its two products are CO₂ and H₂O₂.

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 Microplate Assay Kit is a sensitive assay for determining Oxalate Oxidase activity in various samples. Oxalate Oxidase activity is determined by the product of H₂O₂. 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	

Note:

Dye Reagent: add 1 ml Dye Reagent Diluent to dissolve, then transfer all reagent into Dye Reagent Diluent bottle, mix.

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. 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 10000g 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 10000g 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One Unit of OXO activity is defined as the enzyme produces 1 μmol H_2O_2 per min.

1. According to the volume of sample

$$\begin{aligned}\text{OXO (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &\quad / T \\ &= 0.4 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{OXO (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.4 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cell or bacteria

$$\begin{aligned}\text{OXO (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.4 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

C_{Standard} : the concentration of Standard, 4 mmol/L = 4 $\mu\text{mol/ml}$;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Standard} : the volume of standard, 0.02 ml;

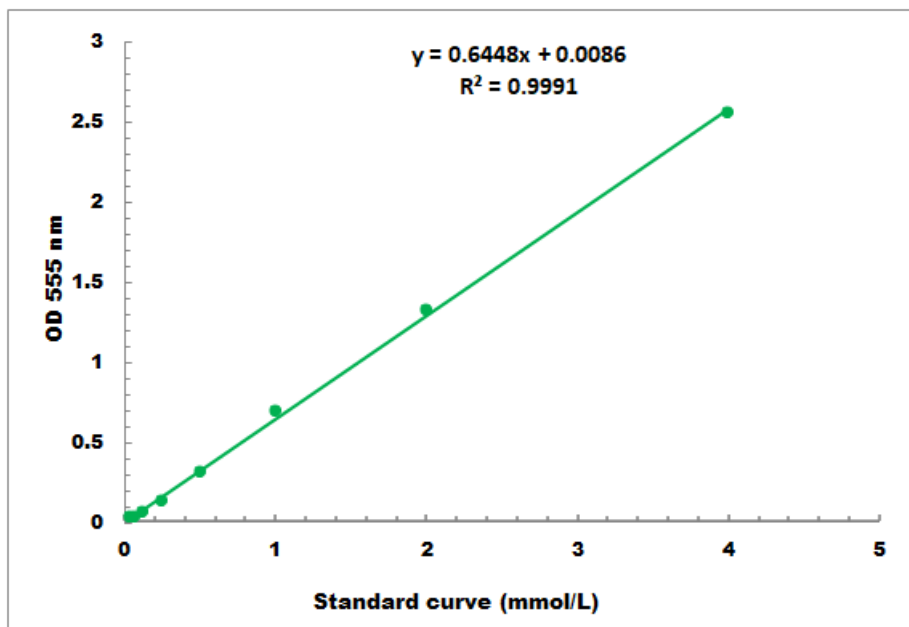
V_{Sample} : the volume of sample, 0.02 ml;

V_{Assay} : the volume of Assay buffer, 1 ml;

T: the reaction time, 10 minutes.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES