Oxalate OxidaseMicroplate Assay Kit

User Manual

Catalog # CAK1182

(Version 1.2C)

Detection and Quantification ofIsocitrate Oxalate Oxidase(OXO) ActivityinTissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

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

oxalate + O_2 + 2 H^+→2 CO_2 + H_2O_2

The 3 substrates of this enzyme are oxalate, O_2, 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 aero-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 Kitis a sensitive assay for determining Oxalate Oxidase activity in various samples.Oxalate Oxidase activity is determined by the product ofH_2O_2. The increase in absorbance at 555 nm is directly proportional to the enzyme activity.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate	2 mlx 1	4 °C
Dye Reagent	Powderx 1	-20 °C, keep in dark
Dye Reagent Diluent	16 ml x 1	4 °C
Standard (4 mmol/L)	1mlx 1	4 °C
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Note:

Dye Reagent: add 1 ml Dye Reagent Diluentto 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 mlAssay 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10000g 4°C for 10minutes, 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 intothe 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:

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 ofOXOactivity is defined asthe enzyme produces 1 μmolH2O2 per min.

1. According to the volume ofsample

$$OXO(U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$OXO(U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$OXO(U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of Standard, 4 mmol/L = 4μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

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

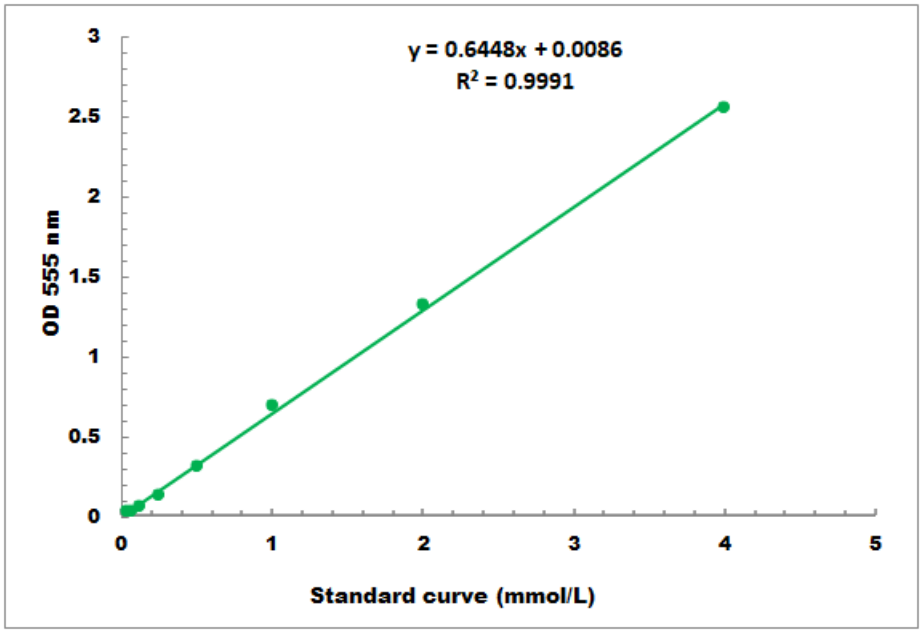
V_{Sample}: the volume of sample, 0.02 ml;

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 towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES