Oxalate oxidase Assay Kit

ARG83396 Oxalate oxidase Assay Kit can be used to measure Oxalate oxidase in Tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83396

Package: 96 wells

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INTRODUCTION

Oxalate oxidase (Mn-OxO) is a Mn-dependent enzyme that catalyzes the oxygen-dependent degradation of oxalate, yielding one mole of H2O2 and two moles of CO2. The active form of Mn-OxO is a hexamer, and each monomer has a canonical cupin fold. The active site is in the center of the β-barrel and contains a Mn ion . Crystallography of Mn-OxO has shown that the coordinating sphere of the enzyme is quite similar to the one found in Mn-SOD. The activity of Mn-OxO is localized to the apoplast, and the enzyme has a dual role in the pathogen defense by inhibiting fungal toxins and promoting lignification through the generation of H2O2 necessary for cross-linking mono-lignols in lignin biosynthesis.

PRINCIPLE OF THE ASSAY

The ARG83396 Oxalate oxidase Assay Kit determined Oxalate oxidase activity by the product of H2O2. The increase in absorbance at 555 nm is directly proportional to the content.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (4 µmol/ml)	4°C
Substrate	2 ml	4°C
Assay Buffer	30 ml x 4	4°C
Reaction Dye	1 vial (lyophilized)	-20°C
Reaction Dye Diluent	16 ml	4°C
Stop Solution	500 µl	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 555 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Reaction Dye at -20°C and protect from light, other component at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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.

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.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- Reaction Dye: Reconstitute the Substrate with 1 ml of Reaction Dye Diluent. Allow the Reaction Dye keep on bench for few minutes. Make sure the Reaction Dye is dissolved completely and mixed thoroughly, then transfer all reagent into Reagent Dye Diluent bottle, mix. Keep the reconstituted the Substrate on ice before use.
- Sample: If the measuring absorbance of samples is higher than the standard, dilute the samples with distilled water before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl** per **samples** into each microplate.
2. Standard wells: Add **10 µl** of **Standard** into microplate.
3. Add **160 µl** of **Reaction Dye** per well into All wells.
4. Mix well.
5. Add **20 µl Substrate** to All wells.
6. Mix well. Incubate at **30°C** for **10 min**.
7. Add **10 µl Stop Solution** to All wells
8. Mix, incubate at **RT** for **2 min**. Read the OD at **555 nm**.

Summary of Oxalate oxidase Assay Kit Procedure

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-	-	10 µl
Reaction Dye	160 µl	160 µl	160 µl
Mix well.			
Substrate	20 µl	20 µl	20 µl
Mix well. Incubate at **30°C** for **10 min**			
Stop Solution	10 µl	10 µl	10 µl
Mix well. Incubate at **RT** oven for **2 min**.			
Read the OD with a microplate reader at **555 nm**.			

CALCULATION OF RESULTS

1. 1. Unit Definition: one unit is defined as the enzyme that generates 1 µmol of oxalate oxidase per minute.

2. Calculate the average absorbance values for each set of samples, standard, positive control, control and blank.

3. Calculation:

A. Definition:

W: the weight of sample, g;

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

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 ml;

$V_{standard}$: the volume of standard sample, 10 µl = 0.01 ml;

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

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

T: the reaction time, 10 minutes.

B. Formula:

a). According to the volume of sample

oxalate oxidase (U/ml) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

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

b). According to the concentration of sample

oxalate oxidase (U/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{total}) \times T]$

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

c). According to the quantity of cells or bacteria

oxalate oxidase ($U/10^4$) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{total}) \times T]$

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

3 Detection range:

The detection range is from 0.04 μmol/ml – 4 μmol/ml.

4. If the samples have been diluted, the calculated concentration must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.