Oxalate Oxidase Assay Kit

Catalog No.: abx298959

Size: 100 Assays

Storage: Store the Dye Reagent in the dark at -20°C. Store all remaining kit components in the dark at 4°C.

Application: For quantitative detection of Oxalate Oxidase activity in tissue homogenates, cell lysates, cell culture media and other biological fluids.

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

Introduction: Oxalate Oxidase, an oxidoreductase enzyme with two known cofactors; FAD and Manganese, catalyses the aerobic oxidation of oxalic acid into Carbon Dioxide (CO_2) and Hydrogen Peroxide (H_2O_2). The latter drives an internal signal transduction cascade which regulates key plant defence mechanisms against stress. Oxalate Oxidase is also implicated to play a role in glyoxylate and dicarboxylate metabolism.

Oxalate Oxidase catalyzes the aerobic oxidation of oxalic acid into Carbon Dioxide (CO_2) and Hydrogen Peroxide (H_2O_2). The concentration of the reaction product (H_2O_2) is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 555 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Dye Reagent: 1 vial
4. Dye Reagent Diluent: 16 ml
5. Standard (4 mmol/L): 1 ml
6. Substrate: 2 ml

Materials Required But Not Provided

1. Microplate reader (555 nm)
2. High-precision pipette and sterile pipette tips
3. Distilled water
4. Mortar
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice
8. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Working Solution**
  Add 1 ml of Dye Reagent Diluent into the Dye Reagent vial and mix thoroughly. Ensure that the Dye Reagent has completely dissolved, then transfer the contents of the Dye Reagent vial into the Dye Reagent Buffer vial to prepare the Dye Reagent Working Solution.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

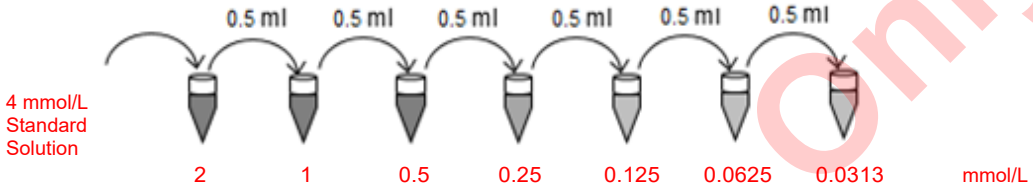
- **Other biological fluids**
  Liquid samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 7 tubes with 2 mmol/L, 1 mmol/L, 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.0625 mmol/L and 0.0313 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of the 4 mmol/L standard solution to the 1st tube (2 mmol/L). Add 0.5 ml of 2 mmol/L standard solution to the 2nd tube (1 mmol/L) and mix thoroughly. Transfer 0.5 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.



2. Set the Sample, Standard and Blank wells on the microplate. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of Sample to the sample well.
4. Add 20 µl of prepared standards to each standard well.
5. Add 20 µl of distilled water to each Blank well.
6. Add 160 µl of Dye Reagent Working Solution to all tubes. Mix thoroughly.
7. Add 20 µl of Substrate to all wells.
8. Mix thoroughly and incubate at 30°C for 10 minutes.
9. Read and record absorbance at 555 nm.

C. Calculations

One unit of Oxalate Oxidase activity is defined as the amount of enzyme required to produce 1 µmol of hydrogen peroxide (H_2O_2) per minute.

Oxalate Oxidase activity per g of sample:

$$\mathbf{Oxalate\ Oxidase\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.4}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Oxalate Oxidase activity per 10^4 cells or bacteria:

$$\mathbf{Oxalate\ Oxidase\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.4}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Oxalate Oxidase activity per ml of sample:

$$\mathbf{Oxalate\ Oxidase\ (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{0.4} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$C_{Standard}$	Concentration of highest standard (4 mmol/L)
T	Reaction time (10 minutes)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
V_{Assay}	Volume of assay buffer (1 ml)
V_{Sample}	Volume of sample (0.02 ml)
$V_{Standard}$	Volume of standard (0.02 ml)