Version 1 Last updated 30 October 2018

ab241007
Oxalate Oxidase Assay Kit

For the measurement of Total Oxalate Oxidase activity in various plant tissues.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Oxalate Oxidase Assay Kit (ab241007) provides a quick and easy method for the measurement of Oxalate Oxidase activity in various samples. In this assay, Oxalate Oxidase converts oxalate into hydrogen peroxide, which in turn, reacts with a probe and converter generating a fluorometric signal (535/587 nm).

The generated fluorescence is directly proportional to the amount of active Oxalate Oxidase present in samples. The assay is simple, sensitive, high–throughput adaptable and can detect less than 4 µU of oxalate Oxidase activity per sample.

The kit allows for the measurement of Oxalate Oxidase activity in plant seeds and plant tissues.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the standard curve using the 0.88 M H_2O_2 standard. Dilute to 10 mM using dH_20.

Create the Reaction Mix, add 50 µl to each well.

Create the Background Mix and add 50 µl to wells containing sample background control.

Measure the plate at Ex/Em = 535/587 nm in kinetic mode at 25°C for 10-60 min.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Briefly centrifuge small vials prior to opening.

Item	Quantity	Storage condition
OxOx Assay Buffer	25 mL	4°C
OxOx Substrate	1 vial	-20°C
OxOx Converter	1 vial	-20°C
Probe (in DMSO)	200 µl	-20°C
OxOx Positive control	1 vial	-20°C
H_2O_2 Standard (0.88 M)	100 µl	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Mortar and Pestle, liquid nitrogen
- Homogenizer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 OxOx Assay Buffer:

Warm OxOx Assay Buffer to room temperature before use. Store at 4°C.

6.2 OxOx Substrate:

Reconstitute each well with 220 µL dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Keep on ice while in use. Use within two months.

6.3 OxOx Converter:

Reconstitute each well with 220 µL dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Keep on ice while in use. Use within two months.

6.4 Red Probe:

Ready to use as supplied. Thaw the probe solution before use, mix well, Store at -20°C. Use within two months.

6.5 OxOx Positive control:

Reconstitute with 100 µL dH_2O. Store at -20°C. Keep on ice while in use. Use within two months.

6.6 H_2O_2 Standard:

Store at -20°C. Keep on ice while in use. Use within two months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 0.88 M H_2O_2 Standard to 10 mM by taking 1 µL of 0.88 M H_2O_2 into 87 µL of dH_2O.

7.2 Then take 5 µL of 10 mM H_2O_2 into 995 µL dH2O to generate 50 pmol/µL H_2O_2.

7.3 Add 0, 2, 4, 6, 8 and 10 µL of the 50 pmol/µL H_2O_2 Standard into a series of wells in 96 well clear plate to generate 0, 100, 200, 300, 400 and 500 pmol/well.

7.4 Adjust volume to 50 µl/well with OxOx Assay Buffer, mix well.

Standard #	50 pmol/µL H_2O_2 standard (µL)	dH_2O (µL)	H_2O_2 concentration Per well (pmol)
1	10	40	1500
2	8	42	400
3	6	44	300
4	4	46	200
5	2	48	100
6	0	50	0

8. Sample Preparation

ΔNote: We suggest using 3-5 different amounts of each sample per well to ensure the readings are within the Standard Curve range and the signal kinetics are within the Linear range.

8.1 For Plant seeds/tissue samples:

- Weight and place plant samples (seeds or tissue;20 mg) into a pre-cooled mortar, carefully add liquid nitrogen and use pestle (placed on dry ice) to break plant cell walls.
- Homogenize ground sample with 200 µL ice-cold OxOx Assay Buffer and incubate for 10 minutes on ice; spin down insoluble material at 10,000 X g for 20 min at 4 °C. Collect the supernatant.
- Use ammonium sulfate precipitation method to remove small molecules that could interfere with the assay.
- Aliquot tissue samples (100 µL) to a clean centrifuge tube, and add 200 µL saturated (4.32M) ammonium sulfate and place on ice for 30 min.
- Spin down samples at 10,000 x g at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume (100 µL) using OxOx Assay Buffer.
- Add 2-40 µL of reconstituted sample into a 96 well clear plate. Adjust volume to 50 µL with OxOx Assay Buffer. For Positive Control: Add 2-20 µL of OxOx Positive Control and adjust final volume to 50 µL with OxOx Assay Buffer.
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  ΔNote: For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

  ΔNote: Parallel wells used as background controls (with sample but without the OxOx substrate) allow for correction of non-specific sample background. Adjust the volume to 50 µl with OxOx Assay Buffer.

9. Assay Procedure

9.1 Make enough reagents for the number of assays to be performed. For each well, prepare 50 µL Reaction Mix containing:

	Reaction Mix	Background Control Mix
OxOx Assay Buffer	45 µL	47 µL
OxOx Converter	2 µL	2 µL
Red Probe	1 µL	1 µL
OxOx Substrate	2 µL	/

9.2 Mix well. Add 50 µL of the Reaction Mix to each well containing the Standard, Positive Control(s), and test samples.

9.3 For samples having high background, add 50 µL of background Control Mix to sample background control well(s). Mix well.

9.4 **Measurement:** Measure the plate at Ex/Em = 535/587 nm in kinetic mode at 25°C for 10-60 min.

ΔNote: We recommend measuring the fluorescence in kinetic mode and choosing two time points (t1 & t2) in the linear range to calculate the OxOx Activity of the samples. The H_2O_2 standard curve can be read in Endpoint mode (i.e., at the end of incubation time).

10. Data Analysis

10.1 Subtract the 0 OxOx Standard reading from all Standard curve readings. Plot the background-subtracted OxOx Standard Curve and calculate the slope.

10.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.

10.3 Calculate the OxOx activity of the test samples. Determine the ΔRFU at linear range of two time points.

10.4 Apply the ΔRFU to the H_2O_2 standard curve to get B pmol of H_2O_2 generated by OxOx at the reaction time (Δt = t2-t1).

Sample OxOx Concentration = B/(t x V) x D pmol/min/µL = µU/µL

Where:

B is the amount of H_2O_2 in the sample well from Standard Curve (pmol).

Δt is Time (min)

V is the sample volume added into the reaction well (µL)

D is the sample dilution factor

ΔNote: Unit Definition: One unit of Oxalate Oxidase is the amount of enzyme that will generate 1.0 µmol of H2O2 per min at pH 5.5 at 25°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

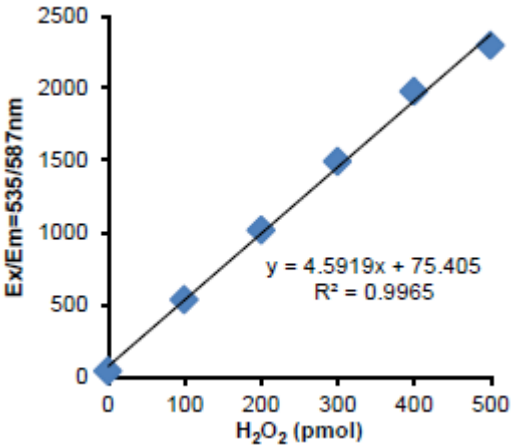

Figure 1. H_2O_2 Standard Curve.

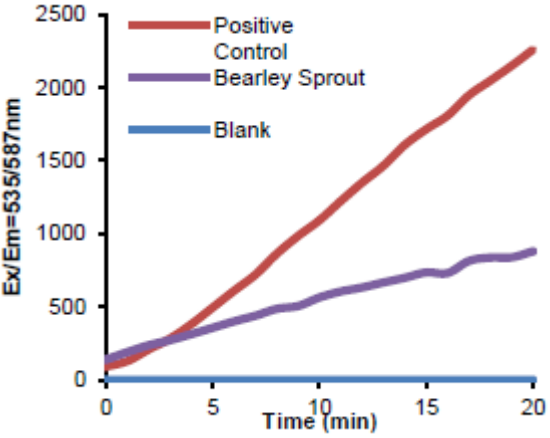

Figure 2. Oxalate Oxidase (OxOx) activity were measured in Barley Seed Lysate (29 µg) and Barley Sprout Lysate (60 µg). Assays were performed following kit protocol.

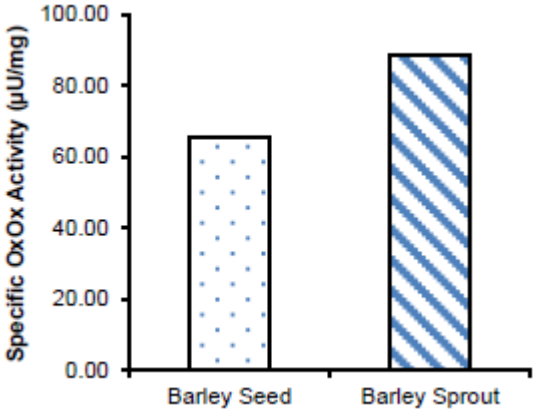

Figure 3. Oxalate Oxidase (OxOx) activity were measured in Barley Seed Lysate (29 µg) and Barley Sprout Lysate (60 µg). Assays were performed following kit protocol.

12. Notes

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