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Oxalate Oxidase Activity Assay Kit (Fluorometric)

rev. 06/17

(Catalog # K509-100; 100 assays; Store at -20°C)

I. Introduction:

Oxalate Oxidase (OxOx EC 1.2.3.4) belongs to the cupin protein superfamily and catalyzes the conversion of Oxalate into H_2O_2 and CO_2. It plays an important role for stress response in plants. For example, wood rotting fungi generates high levels of oxalate, causing plant rot in many crops including lettuce, soybean, dry bean, tomato, etc. Overexpression of OxOx in plants (e.g. tomato, soybean, lettuce and tobacco) is able to generate transgenic plants showing resistance to fungal pathogenesis. Accurate measurement of Oxalate Oxidase activity is valuable for mechanistic studies in plants and development of transgenic plants. BioVision's Oxalate Oxidase Activity Assay kit 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.

$$\text{Oxalate} + H^+ \xrightarrow{\text{Oxalate Oxidase}} H_2O_2 + CO_2 \xrightarrow{\text{Probe + Converter}} \text{Fluorescence detection (Ex/Em = 535/587 nm)}$$

II. Applications:

- Measurement of Oxalate Oxidase activity in various plant tissues
- Analysis of cell signaling pathways such as glyoxylate and dicarboxylate metabolism

III. Sample Type:

- Plant seeds such as barley seeds
- Plant tissues such as barley sprout

IV. Kit Contents:

Components	K509-100	Cap Code	Part Number
OxOx Assay Buffer	25 ml	WM	K509-100-1
OxOx Substrate	1 vial	Blue	K509-100-2
OxOx Converter	1 vial	Green	K509-100-3
OxiRed™ Probe (in DMSO)	200 µl	Red	K509-100-4
OxOx Positive Control	1 vial	Orange	K509-100-5
H_2O_2 Standard (0.88M)	100 µl	Yellow	K509-100-6

V. User Supplied Reagents and Equipment:

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

VI. Storage, Handling and Reagent Preparation:

- **OxOx Assay Buffer:** Warm OxOx Assay Buffer to room temperature before use. Store at 4ºC.
- **OxOx Substrate and 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.
- **OxiRed™ Probe:** Ready to use as supplied. Thaw the probe solution before use, mix well, Store at -20°C. Use within two months.
- **OxOx Positive Control:** Reconstitute with 100 µl dH_2O. Store at -20°C. Keep on ice while in use. Use within two months.
- **H_2O_2 Standard:** Store at -20°C. Keep on ice while in use. Use within two months.

VII. OxOx Activity Assay Protocol:

1. **Sample Preparation:** 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 ºC. 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 (BioVision Cat. # 7096) 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.

   Note:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

   b. 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.

2. **H_2O_2 Standard Curve:** Dilute H_2O_2 Standard to 10 mM by taking 1 µl of 0.88 M H_2O_2 into 87 µl of dH_2O. Then take 5 µl of 10 mM H_2O_2 into 995 µl dH_2O to generate 50 pmol/µl H_2O_2. 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. Adjust volume to 50 µl/well with OxOx Assay Buffer, mix well.
3. **Assay Development Reaction:** 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
OxiRed™ Probe	1 µl	1 µl
OxOx Substrate	2 µl	--

Mix well. Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control(s), and test samples.
*For samples having high background, add 50 µl of background Control Mix to sample background control well(s). Mix well.

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

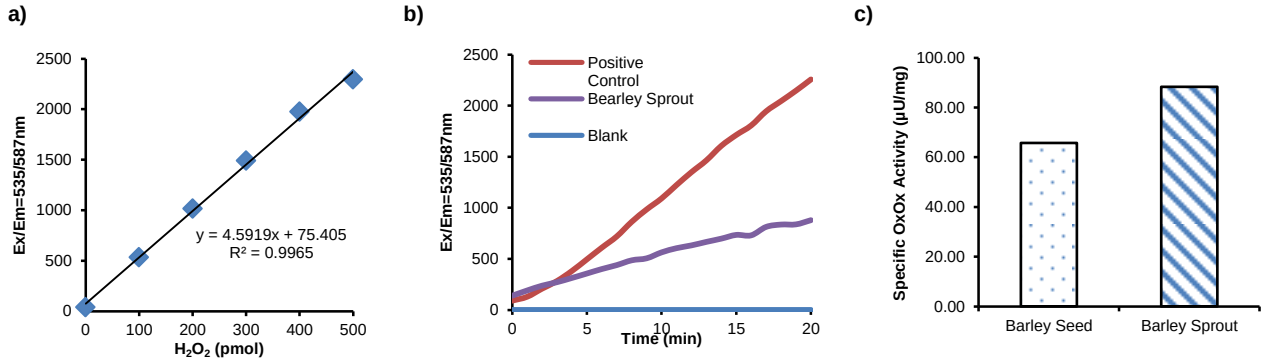
Note: We recommend measuring the fluorescence in kinetic mode, and choosing two time points (t_1 & t_2) in the linear range to calculate the OxOx Activity of the samples. The H_2O_2 standard curve can be read in Endpoint mode (i.e., at the end of incubation time).

5. **Calculation:** Subtract the 0 standard reading from all readings. Plot the H_2O_2 standard curve. If sample background readings are high, subtract the background control reading from sample readings. Calculate the OxOx activity of the test samples. Determine the ΔRFU (ΔRFU = RFU_2- RFU_1) at linear range of two time point ($t_2 - t_1$). 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 Activity = B/(t X V) x D = pmol/min/µl = µU/µl

Where: **B** = H_2O_2 amount from the standard curve (pmol).
Δt = time (min).
V = sample volume added into the reaction well (µl).
D = Sample dilution factor

Unit Definition: One unit of Oxalate Oxidase is the amount of enzyme that will generate 1.0 µmol of H_2O_2 per min at pH 5.5 at 25°C.



Figures. A: H_2O_2 Standard Curve using this assay protocol. **B and C:** Oxalate Oxidase (OxOx) activity were measured in Barley Seed Lysate (29 µg) and Barley Sprout Lysate (60 µg). Assays were performed following kit protocol.

VIII. RELATED PRODUCTS

Glucose and Sucrose Assay (K616-100)
Glucose Uptake Colorimetric Assay (K676-100)
Glucose Uptake Fluorometric Assay (K666-100)
Maltose and Glucose Assay (K618-100)
Glucose-6-Phosphate dehydrogenase Activity Assay (K757-100)
Glucose Dehydrogenase Activity Assay (K786-100)
Oxalate Decarboxylase Colorimetric Assay (K664-100)
Glucose-6-Phosphate Assay (K657-100)
Glucose Assay kit II (K686-100)
Oxalate (Oxalic Acid) Colorimetric Assay (K663-100)
Ascorbic Acid Colorimetric Assay II (K671-100)
Ascorbic Acid Colorimetric Assay (K661-100)

FOR RESEARCH USE ONLY! Not to be used on humans.