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Oxalate Decarboxylase Activity Colorimetric Assay Kit

03/14

(Catalog # K664-100; 100 assays; Store at -20°C)

I. Introduction:

Oxalate Decarboxylase (OXDC, EC 4.1.1.2) belongs to the cupin superfamily and is composed of two β-barrel domains. OXDC catalyzes the conversion of Oxalate into Formate and CO_2 and plays an important role in stress response. In humans, high levels of oxalate can lead to various health problems including hyperoxaluria, kidney stones, and renal failure. Wood rotting fungi generates high levels of oxalate, causing rot in many crops including lettuce, soybean, dry bean, and tomato etc. Recent studies show that overexpression of OXDC in plants (e.g. tomato, soybean, lettuce, and tobacco) results in transgenic plants with resistance to fungal pathogenesis. Accurate measurement of Oxalate Decarboxylase activity is useful for a variety of therapeutic, diagnostic, and mechanistic studies. BioVision's Oxalate Decarboxylase Activity Assay kit provides a quick and easy way to measure Oxalate Decarboxylase activity in various samples. In this assay, Oxalate Decarboxylase converts oxalate to formate, which subsequently converts a nearly colorless probe to a colored product with strong absorbance at 450 nm. The assay is simple, sensitive, high-throughput adaptable and can detect less than 20 µU of oxalate decarboxylase activity in a variety of samples.

$$\text{Oxalate} + H^+ \xrightarrow{\text{OXDC}} \text{Formate} + CO_2 \xrightarrow{\text{OXDC Enzyme Mix}} \text{Intermediate} \xrightarrow{\text{Probe}} \text{Color detection (OD 450 nm)}$$

II. Application:

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

III. Sample Type:

- Fungi such as mushroom
- Plant tissues

IV. Kit Contents:

Components	K664-100	Cap Code	Part Number
OXDC Assay Buffer I	20 ml	WM	K664-100-1
OXDC Assay Buffer II	15 ml	NM	K664-100-2
OXDC Substrate (Lyophilized)	1 vial	Blue	K664-100-3
OXDC Enzyme Mix (Lyophilized)	1 vial	Green	K664-100-4
OXDC Probe (Lyophilized)	1 vial	Red	K664-100-5
OXDC Positive Control	0.1 ml	Orange	K664-100-6
Formate Standard (100 mM)	0.1 ml	Yellow	K664-100-7

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **OXDC Assay Buffer I and II:** Bring OXDC Assay Buffer I and II to room temperature before use. Store at either 4°C or -20°C.
- **OXDC Substrate:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **OXDC Enzyme Mix:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **OXDC Probe:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **OXDC Positive Control:** Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Formate Standard:** Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VII. OXDC Activity Assay Protocol:

1. **Sample Preparation:** Pre-cool mortar and pestle in an ice bucket containing dry ice. Grind the plant tissue or fruit (~10 mg) in pre-cooled mortar and pestle to break the cell wall. Homogenize with 100 µl ice cold OXDC Assay Buffer I and incubate for 10 min. on ice. Centrifuge at 10,000 X g for 5 min. & collect the supernatant.

   Note:

   While preparing the samples, we recommend adding Protease Inhibitor Cocktail (Cat # K271-500) at a 1:1000 ratio.

2. **OXDC Assay:** Add 1-10 µl of sample supernatant into desired well in a 96-well plate. For Positive Control, add 2-15 µl of OXDC Positive Control into desired well(s). Add 2 µl of OXDC Substrate into Positive Control & sample wells and adjust the volume to 25 µl with OXDC Assay Buffer I. Preincubate the plate at 37°C for 1 hr. After 1 hr. incubation, add 25 µl of OXDC Assay Buffer II.

   Notes:

   a. For unknown samples, we suggest doing a pilot experiment & testing several doses to ensure the readings are within the Standard

Curve range.

b. For samples having high formate background, prepare parallel sample well(s) as background control(s) with same volume of sample as test wells but without the OXDC substrate. Add 25 µl of OXDC Assay Buffer I & 25 µl of OXDC Assay Buffer II.

c. One hr. preincubation time (T) used to measure the OXDC activity of samples is based upon our experience with typical concentrations of OXCD in samples tested. This may be increased or decreased depending upon OXDC activity in your samples.

3. **Formate Standard Curve:** Dilute Formate Standard to 1 mM by adding 10 µl of 100 mM Formate into 990 µl of dH_2O. Add 0, 2, 4, 6, 8 and 10 µl of the 1 mM Formate Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol Formate/well. For Formate Standards & Development reaction (step 4), freshly prepare OXDC Buffer Mix in a separate tube by adding equal volume of OXDC Assay Buffer I and OXDC Assay Buffer II. Mix properly. Adjust the volume of Formate Standards to 50 µl/well with OXDC Buffer Mix.

   Note: Don't store the Buffer mix.

4. **Development Reaction:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
OXDC Buffer Mix	46 µl
OXDC Enzyme Mix	2 µl
OXDC Probe	2 µl

   Mix well. Add 50 µl of Reaction Mix to each well containing Samples, Standards, Positive Control(s) and Background Control(s). Incubate for 40 min. at 37°C

   Note: Measurement of OXDC activity is a 2-step enzymatic assay and the development reaction incubation time does not indicate the activity of the enzyme.

5. **Measurement:** Measure the absorbance (OD 450 nm).

6. **Calculation:** Subtract 0 Standard reading from all readings. Plot Formate Standard Curve. If sample background control reading is high, subtract the background control reading from sample reading. Apply corrected OD to Formate Standard Curve to get B nmol of Formate generated by OXDC at a given time. Calculate the OXDC activity of the test samples.

Sample OXDC Activity = B/(T X V) x D = nmol/min/ml = mU/ml

Where: **B** is formate amount in the sample well from the Standard Curve (nmol)
T is time (min.) [preincubation time]
V is sample volume added into the reaction well (ml)
D is Sample dilution factor

OXDC activity can also be expressed as mU/mg of sample.

Unit Definition: One unit of Oxalate Decarboxylase is the amount of enzyme that generates 1.0 µmol of Formate per min. at pH 5 at 37°C.

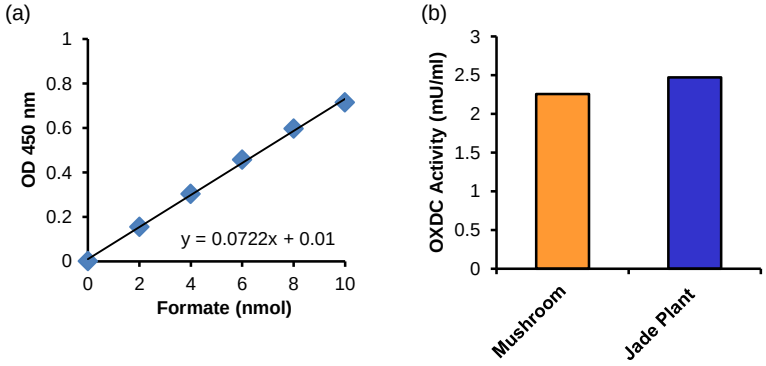

Figure: Formate Standard Curve (a). Oxalate Decarboxylase (OXDC) activity was measured in white mushroom lysate (20 µl), and Jade Plant lysate (20 µl). Assay was performed following the kit protocol.

VIII. RELATED PRODUCTS:

Oxalate (Oxalic Acid) Colorimetric Assay Kit (K663)
Hydroxyproline Colorimetric Assay Kit (K555)
Lactate Dehydrogenase (LDH) Activity Assay Kit (K726)
Ascorbic Acid Colorimetric/Fluorometric Assay Kit (K661)
Ascorbic Acid Colorimetric Assay Kit II (FRASC) (K671)
Protease Inhibitor Cocktail (K271-500)

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