ab196993

Oxalate Decarboxylase Activity Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Oxalate Decarboxylase activity in a variety of samples.

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

Version 1 Last Updated 24 March 2015

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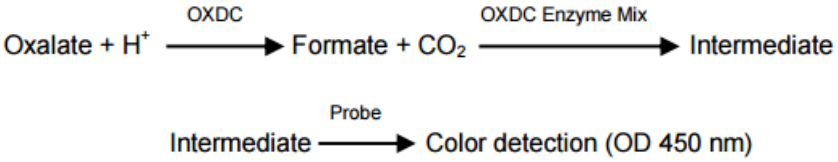
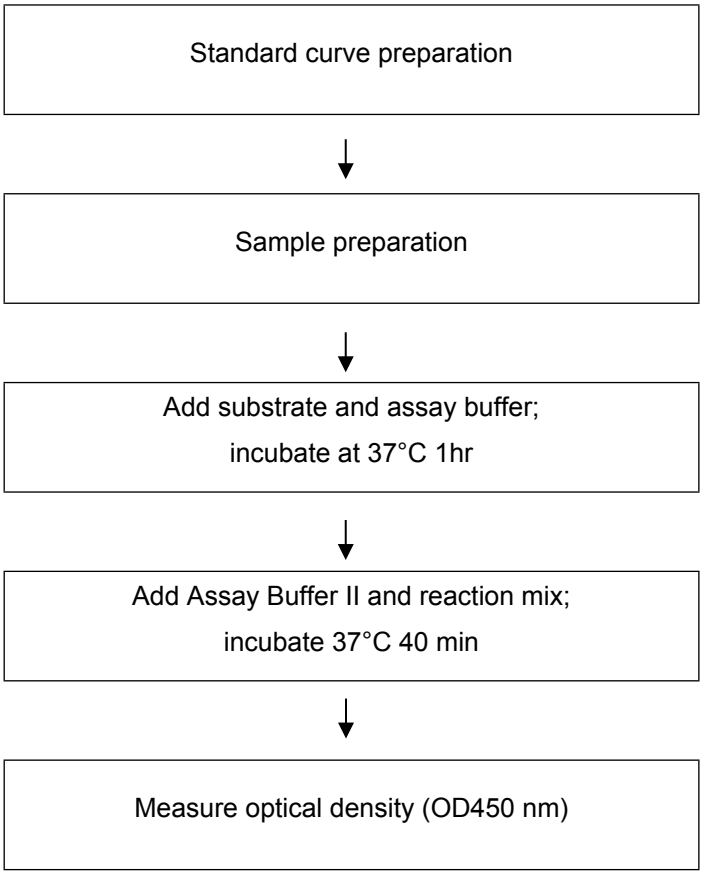
1. BACKGROUND

Oxalate Decarboxylase Activity Assay kit (colorimetric) (ab196993) provides a quick and easy way to measure oxalate decarboxylase activity in a variety of 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-thouroughput adaptable and can detect less than 20 µU of oxalate decarboxylase activity in a variety of samples.

Oxalate Decarboxylase (OXDC) belongs to the cupin superfamily and is composed of two β-barrel domains. OXDC catalyzes the conversion of oxalate into formate and CO2 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.



2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
OXDC Assay Buffer I	20 mL	-20°C	4°C / -20°C
OXDC Assay Buffer II	15 mL	-20°C	-20°C
OXDC Substrate (lyophilized)	1 vial	-20°C	-20°C
OXDC Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
OXDC Probe (lyophilized)	1 vial	-20°C	-20°C
Positive Control	100 µL	-20°C	-20°C
Formate Standard (100 mM)	100 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plates for colorimetric assay
- Heat block or water bath
- Dounce homogenizer or pestle
- Protease Inhibitor Cocktail (ab65621)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **OXDC Assay Buffer I:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **OXDC Assay Buffer II:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C

9.3 **OXDC Substrate:**

Reconstitute in 220 µL ddH_2O. Pipette up and down to dissolve completely. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 **OXDC Enzyme Mix:**

Reconstitute in 220 µL ddH_2O. Pipette up and down to dissolve completely. Aliquot enzyme mix so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use

9.5 **OXDC Probe:**

Reconstitute in 220 µL ddH_2O. Pipette up and down to dissolve completely. Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light. Once the probe is thawed, use within two months.

9.6 **OXDC Positive Control:**

Ready to use as supplied. Aliquot positive control so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

9.7 **Formate Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Freshly prepare OXDC Buffer Mix in a separate tube by adding equal volume of OXDC Assay Buffer I and OXDC Assay Buffer II. Initial recommendation: 1 mL = 500 µL Buffer I + 500 µL Buffer II.

10.2 Prepare a 1 mM Formate standard by diluting 10 µL of the provided 100 mM Formate standard with 990 µL of dH_2O.

10.3 Using 1 mM formate standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	OXDC Buffer Mix (µL)	Final volume standard in well (µL)	End Conc. OXDC in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Tissue (Plant, Fruit or Fungi):

11.1.1 Harvest the amount of plant tissue or fruit necessary for each assay (initial recommendation = 10 mg).

11.1.2 Grind in pre-cooled mortar with pestle to break the cell wall until a paste is formed from grinding (for the most efficient extraction, quickly freeze under liquid nitrogen and grind in a pre-cooled mortar using a pestle).

While preparing samples, we recommend adding Protease Inhibitor Cocktail (ab65621) at 1:1000 ratio.

11.1.3 Add 100 µL of ice-cold OXDC Assay Buffer I to tissue/fruit paste/powder and homogenize.

11.1.4 Incubate the homogenate for 10 minutes on ice and centrifuge at 10,000 x *g* for 5 minutes.

11.1.5 Collect supernatant.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 10 µL samples.
- Background control sample wells= 1 – 10 µL samples.
- Positive control = 2 – 15 µL Positive control (Section 9.6).

12.2 Add 2 µL of OXDC Substrate into positive control and sample wells and adjust the volume to 25 µL with OXDC Assay Buffer I.

12.3 For background control sample wells, add 25 µL of OXDC Assay Buffer I.

The table below summarizes the reaction wells set up:

Component	Sample well (µL)	Positive control well (µL)	Background well (µL)
Sample	**1 – 10**		**1 – 10**
Positive control		**2 – 15**	**0**
OXDC Substrate	**2**	**2**	**0**
OXDC Assay Buffer I	**Up to 25**	**Up to 25**	**Up to 25**

12.4 Pre-incubate for 1 hour at 37°C. ***NOTE:*** *One hour pre-incubation time recommendation is based on our experience with typical concentrations of OXCD in samples tested. The pre-incubation time may be increased or decreased depending upon OXDC activity in your samples.*

12.5 After 1 hour incubation, add 25 µL of OXDC Assay Buffer II to all samples, positive and background wells.

12.6 **Oxalate Decarboxylase Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction, using the OXDC Buffer Mix prepared in section 10.1:

Component	Reaction Mix (µL)
OXDC Buffer Mix (Buffer I + Buffer II)	**46**
OXDC Enzyme Mix	**2**
OXDC Probe	**2**

Mix enough reagents for the number of assays (samples, standards, positive control and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards + positive control + background control sample + 1).

12.7 Add 50 µL of Reaction Mix into each sample, standard, positive control and background control sample wells.

12.8 Mix and incubate at 37°C for 40 minutes protected from light.

12.9 Measure absorbance at OD=450 nm on a microplate reader.

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.*

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Formate.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$B = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 OXDC activity (in nmol/min/mL or mU/mL) in the test samples is calculated as:

$$OXDC\ Activity = \left(\frac{B}{T\ x\ V}\right) * D$$

Where:

B = Amount of Formate from Standard Curve (nmol).

V = Sample volume added into the reaction well (mL).

T = time (min) [pre-incubation time from Section 12.4]

D = Sample Dilution factor.

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

Unit definition:

1 Unit OXDC activity = amount of Oxalate Decarboxylase that generates 1.0 µmol of Formate per minute at pH5 at 37°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

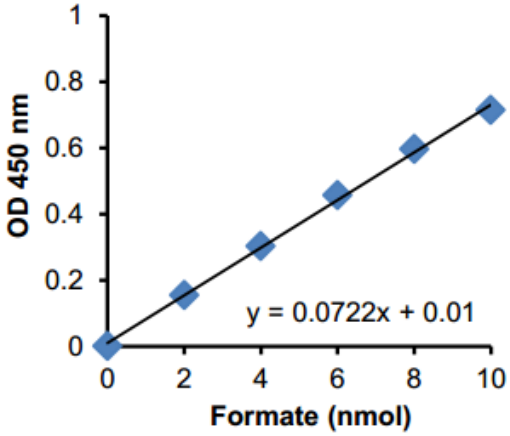


Figure 1. Typical formate standard calibration curve using colorimetric reading.

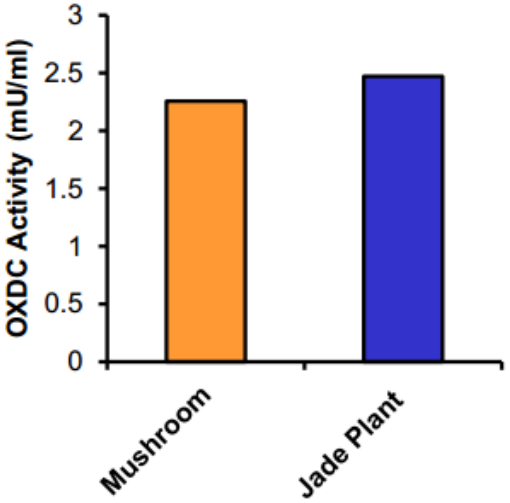


Figure 2: Oxalate Decarboxylase (OXDC) activity was measured in white mushroom lysate (20 µL), and jade plant lysate (20 µL).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare buffers, substrate, enzyme mix, probe, positive control and standard (aliquot if necessary); get equipment ready.
- Prepare standard curve using OXDC Buffer Mix.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up wells:

Component	Sample (µL)	Background Control (µL)	Positive Control (µL)
Sample	**1 -10**	**1 – 10**	**2 -15**
Substrate	**2**	**0**	**2**
Assay Buffer I	**Adjust volume to 25 µL**	**Adjust volume to 25 µL**	**Adjust volume to 25 µL**
Pre-incubate 37°C 1 hour			
Assay Buffer II	**25**	**25**	**25**

- Prepare OXDC Reaction Mix (Number samples + standards + background control + positive control + 1).

Component	Colorimetric Reaction Mix (µL)
OXDC Buffer Mix	**46**
OXDC Enzyme Mix	**2**
OXDC Probe	**2**

- Add 50 µL OXDC Reaction Mix to the standard, sample, background control and positive control wells.
- Incubate plate at 37°C 40 mins protected from light.
- Measure plate at OD 450 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. **FAQ**

18. **INTERFERENCES**

19. **NOTES**

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