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Product Information

Oxalate Decarboxylase Activity Assay Kit

Catalog Number **MAK214**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Oxalate Decarboxylase (OXDC, EC 4.1.1.2) catalyzes the conversion of oxalate to formate and carbon dioxide in the presence of molecular oxygen.[1,2] OXDC-responsive proteins are involved in the regulation of metabolism and stress responses in plants.[2]

The Oxalate Decarboxylase Activity Assay Kit provides a simple and high throughput adaptable procedure for measuring OXDC activity in a variety of plant and fungal tissues. OXDC activity is determined by generating a colorimetric product with absorbance at 450 nm (A_{450}) proportional to the enzymatic activity present. One unit of oxalate decarboxylase is the amount of enzyme required to generate 1.0 μmole of formate per minute at pH 5 at 37 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

OXDC Assay Buffer I Catalog Number MAK214A	20 mL
OXDC Assay Buffer II Catalog Number MAK214B	15 mL
OXDC Substrate Catalog Number MAK214C	1 vl
OXDC Enzyme Mix Catalog Number MAK214D	1 vl
OXDC Probe Catalog Number MAK214E	1 vl
OXDC Positive Control Catalog Number MAK214F	0.1 mL
Formate Standard, 100 mM Catalog Number MAK214G	0.1 mL

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Mortar and pestle
- Protease Inhibitor Cocktail (Catalog Number P9599 or equivalent, optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

OXDC Assay Buffer I and OXDC Assay Buffer II – Store at –20 °C or 2–8 °C. Allow buffers to come to room temperature before use.

OXDC Buffer Mix – Mix equal volumes of OXDC Assay Buffer I and OXDC Assay Buffer II. Prepare fresh before each use.

OXDC Substrate – Reconstitute with 220 μL of water. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.

OXDC Enzyme Mix – Reconstitute with 220 μL of water. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.

OXDC Probe – Reconstitute with 220 μL of water. Mix well by pipetting. Aliquot and store at –20 °C. Use within 2 months.

OXDC Positive Control and Formate Standard – Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Formate Standards for Colorimetric Detection
Dilute 10µL of 100 mM (100 nmole/µL) Formate Standard Solution with 990 µL of water and mix well to prepare a 1 mM (1 nmole/µL) Formate Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM (1 nmole/µL) Formate Standard Solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8 and 10 nmole/well standards. Add OXDC Buffer Mix to each well to bring the volume to 50 µL.

Sample Preparation
Cool a mortar and pestle on dry ice. Prepare plant tissue by grinding with the mortar and pestle to break down cell wells. Homogenize 10 mg with 100 µL of ice cold OXDC Assay Buffer I. Keep on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.
Note: Addition of a protease inhibitor cocktail during homogenization is recommended.

Add 1–10 µL of the sample supernatant into duplicate wells. Bring samples to a final volume of 15 µL using OXDC Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, especially background caused by formate in the sample, include a Sample Blank for each sample by omitting the OXDC Substrate. The Sample Blank readings can then be subtracted from the sample readings.

For a positive control (optional), add 2–15 µL of the OXDC Positive Control solution to the desired wells. Adjust the final volume to 15 µL with the GPDH Assay Buffer.

Assay Reaction
1. Set up Enzymatic Reaction Mixes according to the scheme in Table 1. 10 µL of Enzymatic Reaction Mix is required for each reaction (well).

Table 1.
Enzymatic Reaction Mixes

Reagent	Controls and Samples	Sample Blank
OXDC Assay Buffer I	8 µL	10 µL
OXDC Substrate	2 µL	–

2. Add 10 µL of the appropriate Enzymatic Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 37 °C for 60 minutes.
Note: Incubation time depends on the activity of OXDC in the samples.

4. After the incubation, add 25 µL OXDC Assay Buffer II to each well to stop the enzymatic reaction. Record the incubation time in minutes.

5. Set up a Development Reaction Mix according to the scheme in Table 2. 50 µL of Development Reaction Mix is required for each reaction (well).

Table 2.
Development Reaction Mix

Reagent	Standards, Samples, Controls, and Sample Blank
OXDC Buffer Mix	46 µL
OXDC Enzyme Mix	2 µL
OXDC Probe	2 µL

6. Add 50 µL of the Development Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

7. Incubate the plate at 37 °C for 40 minutes.

8. Measure the absorbance (A_{450}) in a microplate reader.

Results

Calculations

Correct for the background by subtracting the measurement obtained for the 0 (blank) Formate Standard from that of the standards, controls, and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Formate Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank ΔA_{450} value from the Sample ΔA_{450} reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of formate (nmole/well) generated by the OXDC assay.

OXDC activity:

$$\text{OXDC Activity} = \frac{S_a}{(\text{Reaction Time}) \times S_v}$$

where:

S_a = Amount of formate (nmole) generated in unknown sample well during the Enzymatic Reaction from standard curve

Reaction Time = length of Enzymatic Incubation (minutes)

S_v = sample volume (mL) added to well

OXDC activity is reported as nmole/min/ mL = milliunit/mL.

Unit definition: One unit of oxalate decarboxylase is the amount of enzyme required to generate 1.0 μmole of formate per minute at pH 5 at 37 °C.

Sample Calculation:

Amount of formate (S_a) = 5.84 nmole (from standard curve)

Incubation time = 60 minutes

Sample volume (S_v) = 0.050 mL

OXDC activity in sample well:

$$\text{nmole/min/mL (milliunits/mL)} = \frac{5.84 \text{ nmole/well}}{(60 \text{ min}) \times 0.050 \text{ mL /well}} = 1.95$$

References

1. Karmakar, T. et al., CO_2 migration pathways in oxalate decarboxylase and clues about its active site. J. Phys. Chem. B., **117**, 12451–12460 (2013).
2. Chakraborty, N. et al., Reduction of oxalate levels in tomato fruit and consequent metabolic remodeling following overexpression of a fungal oxalate decarboxylase. Plant Physiol., **162**, 364–378 (2013).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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