

Product Information

Oxalic Acid Colorimetric Assay Kit

Catalog Number **MAK179**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

Oxalic acid (Oxalate) is a strong dicarboxylic acid that occurs in several plants and vegetables. In animals, it is either incorporated from the diet or produced during ascorbic acid and glyoxylic acid metabolism. Oxalic acid is not metabolized and is excreted in the urine. Hence, it can act as a marker for glyoxylic and ascorbic acid metabolism. Furthermore, oxalic acid has been studied as a pathogenic factor for *Sclerotinia sclerotiorum* that inhibits the oxidative burst response in host plants.¹ It is also known to alleviate chilling injury in peach fruit.² Elevated levels of oxalic acid (oxalate) have been associated with the formation of kidney stones. Hence, oxalate measurements can be used for the diagnosis and monitoring of renal stones.

The Oxalic Acid Assay Kit provides a simple and sensitive procedure for measuring oxalic acid in variety of samples including animal and plant tissue. In this assay, oxalic acid concentration is determined by a coupled enzyme reaction, which results in a colorimetric (450 nm) product, proportional to the oxalate present.

Components

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

Oxalate Assay Buffer Catalog Number MAK179A	25 mL
Oxalate Developer Buffer Catalog Number MAK179B	15 mL
Oxalate Converter Catalog Number MAK179C	0.2 mL
Oxalate Enzyme Mix Catalog Number MAK179D	1 vL
Oxalate Probe Catalog Number MAK179E	1 vL
Oxalate Standard Catalog Number MAK179F	1 vL

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
- Activated charcoal (>80 mesh)
- Mortar and pestle

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.

Oxalate Assay and Oxalate Development Buffers – Allow buffers to come to room temperature before use.

Oxalate Converter – Aliquot and store protected from light at $-20\text{ }^{\circ}\text{C}$. Use within two months.

Oxalate Enzyme Mix and Oxalate Probe – Reconstitute each with 220 μL of water. Mix well by pipetting. Aliquot each and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution and keep on ice while in use.

Oxalate Standard – Reconstitute with 100 μL of water to generate 100 mM (100 nmole/ μL) Oxalate Standard solution. Store protected from light at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution and keep on ice while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at $-20\text{ }^{\circ}\text{C}$, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

Oxalate Standards for Colorimetric Detection

Dilute 10 μL of the 100 mM (100 nmole/ μL) Oxalate Standard Solution with 990 μL of water to prepare a 1 mM (1 nmol/ μL) standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM Oxalate standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmol/well standards. Add Oxalate Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

Plant tissue or fruits (~15 mg) should be ground in a pre-cooled mortar with pestle till a paste is formed. For the most effective extraction, tissue should be rapidly frozen in liquid nitrogen and ground with a pre-cooled mortar and pestle to form a paste or powder.

Plant tissue paste (or powder) or should then be rapidly homogenized in 150 μL of cold Oxalate Assay buffer. Incubate the homogenate for 10 minutes on ice and centrifuge at $10,000 \times g$ for 5 minutes to remove insoluble material.

Animal tissue or cells should be homogenized directly in cold Oxalate Assay Buffer and centrifuged at $10,000 \times g$ for 5 minutes to remove insoluble material.

Add 1–50 μL of the supernatant into a 96 well plate and bring the volume to 50 μL with Oxalate Assay Buffer.

Serum and other liquid samples can be directly added to the wells.

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 urine samples, it is suggested to collect the sample (10 ml, 24 hours prior to testing) in a bottle containing 10 ml of 10 M hydrochloric acid. Centrifuge at $1,000 \times g$ for 10 minutes and either use the supernatant immediately for analysis or store at $-20\text{ }^\circ\text{C}$.

To reduce background in urine samples, it is recommended to purify the urine samples further by mixing 1 mL of urine with activated charcoal (25 mg) for 5 minutes at room temperature. Centrifuge at $10,000 \times g$ for 5 minutes and use 10 μL of the supernatant for the assay.

Note: To correct for sample interference (e.g., urine sample), it is suggested to do samples in pairs.

Estimate sample background by adding 4 μL of 1 mM Oxalate Standard to one of each sample pair as an internal standard (Sample_{spiked}).

Conversion

Add 2 μL of Oxalate Converter to each Standard and Sample well. Mix and incubate at $37\text{ }^\circ\text{C}$ for 1 hour.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
MasterReaction Mix

Reagent	Samples and Standards
Oxalate Development Buffer	46 μL
Oxalate Enzyme Mix	2 μL
Oxalate Probe	2 μL

2. Add 50 μL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at $37\text{ }^\circ\text{C}$. Protect the plate from light during the incubation.
3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Oxalate Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Oxalate standards to plot a standard curve.

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

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Oxalate present in the sample may be determined from the standard curve.

Concentration of Oxalate (Non-spiked samples)

$$C (\mu\text{M}) = S_a/S_v \times \text{Sample Dilution Factor}$$

S_a = Amount of Oxalate in unknown sample (nmole) from standard curve

S_v = Sample volume (mL) added into the wells

C = Concentration of Oxalate in sample

Oxalate Molecular Weight: 90.03 g/mole

Concentration of Oxalate (Spiked samples)

Note: For samples with interference, correct sample interference for the sample reading using the following equation:

$$S_a = \frac{(A_{450})_{\text{sample}}}{(A_{450})_{\text{sample}_{\text{spiked}}} - (A_{450})_{\text{sample}}} \times 4 \text{ nmole}$$

S_a = Amount of Oxalate in unknown sample (nmole)

$(A_{450})_{\text{sample}}$ = absorbance reading for sample

$(A_{450})_{\text{sample}_{\text{spiked}}}$ = absorbance reading for spiked sample

References

1. Cessna, S.G. et al., Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. *Plant Cell*, **12(11)**, 2191-2200 (2000).
2. Jin, P. et al., Oxalic acid alleviates chilling injury in peach fruit by regulating energy metabolism and fatty acid contents. *Food Chem.*, **161**, 87-93 (2014).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice 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 samples will be used 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 Master Reaction Mix 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 mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	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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