CheKine™ Mirco Glycolate Oxidase (GO) Activity Assay Kit

Cat #: KTB1902 Size: 48 T/48 S 96 T/96 S

	Mirco Glycolate Oxidase (GO) Activity Assay Kit		
REF	**Cat #:** KTB1902	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glycolate oxidase (EC 1.1.3.15) is an enzyme involved in the glycolate cycle and is also a key enzyme in plant photorespiratory metabolism. It catalyzes the oxidation of glycolate to glyoxylate. By measuring glycolate oxidase activity, one can understand the basic methods of plant photosynthetic and respiratory metabolism. CheKine™ Mirco Glycolate Oxidase (GO) Activity Assay Kit provides a simple, convenient, and rapid method for detecting glycolate oxidase activity, suitable for plant tissue samples. The principle of the kit is based on the oxidation of glycolate by glycolate oxidase to form glyoxylate, which reacts with phenylhydrazine hydrochloride to form glyoxylate phenylhydrazone, exhibiting a characteristic absorption peak at 324 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	8 mL	16 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	1.25 mL	2.5 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 324 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Analytical balance, low-temperature centrifuge, ice maker
- Deionized water
- Homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent II: Prepared before use. Add 2.5 mL of deionized water to 48 T and 5 mL of deionized water to 96 T, dissolve thoroughly, and keep for future use. Any unused reagents can be aliquoted and stored at -20℃ in the dark for up to 1 week.

Note: Extraction Buffer and Working Reagent II are toxic and have a strong odor. It is recommended to perform the experiment in a fume hood.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: Use fresh samples whenever possible. If not assayed immediately, samples can be stored at -80℃ for 2 weeks. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Plant Tissue: Weigh approximately 0.1 g of sample, add 1 mL of Extraction Buffer, and grind to homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃, collect the supernatant, and place on ice for subsequent measurement.

Note: 1. For samples with high pigment content, 5 mg of activated charcoal can be added during enzyme extraction for adsorption.

2. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 324 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in a 96-well UV plate or microquartz cuvettes):

Reagent	Test Well (μL)
Sample supernatant	10
Reagent I	130
Working Reagent II	40
Reagent III	20
Mix thoroughly and immediately measure the absorbance A at 324 nm at 10 s and 190 s in a 96-well UV plate or microquartz cuvettes; Calculate $\Delta A=A_2-A_1$.	

Note: 1. Before conducting the experiment, it is recommended to perform a pilot test with 2-3 samples expected to show significant differences. If ΔA is less than 0.001, appropriately extend the reaction time (e.g., measure absorbance at 310 s and 610 s) or increase the sample size. If A_1 is greater than 1.0, the sample can be further diluted with Extraction Buffer, and the final result should be multiplied by the dilution factor, or reduce the amount of sample used for extraction.

2. If multiple samples need to be measured, prepare the working solutions of the above reagents proportionally according to the experimental requirements.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plate calculation formula as below

Calculated by sample fresh weight

Active unit definition: The amount of enzyme required to oxidize 1 nmol of glycolate per min per g of tissue.

GO (U/g fresh weight)=ΔA÷(ε×d)×10^9×V_{Total}÷(V_{Sample}×Cpr÷$V_{Total\ Sample}$)÷T**=784.31×ΔA÷W**

Where: ε: Millimolar extinction coefficient of glyoxylate phenylhydrazone: 17,000 L/mmol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9: conversion factor, 1 mol=10^9 nmol; V_{Total}: total reaction volume, 2×10^{-4} L; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; W: sample fresh weight, g; T: reaction time, 3 min.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

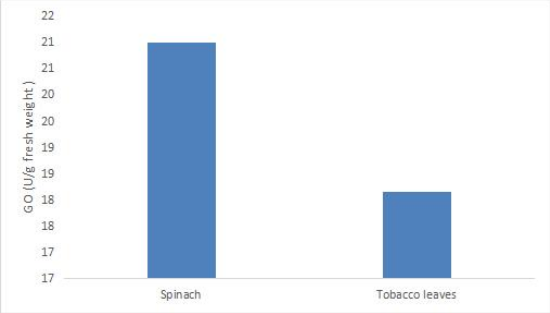

Figure 1. Determination GO Activity in Spinach and Tobacco leaves by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.