（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K692-S

Specification：50 Assays(48 samples)/100 Assays(98 samples)

Measuring instrument：Spectrophotometer（324 nm）

Detection range：0.3-350 U/mL

Elabscience®Glycolate Oxidase Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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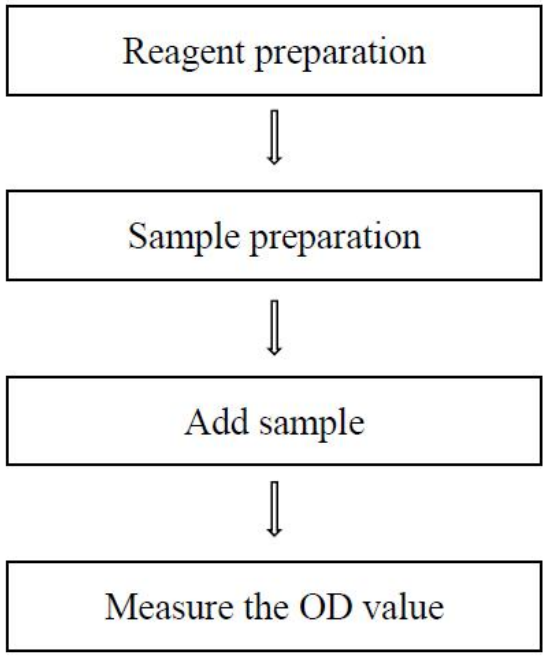
Assay summary



Intended use

This kit can be used to measure glycolate oxidase activity in plant tissue sample.

Detection principle

Glycolate oxidase catalyzes sodium glycolate substrate to form glyoxylic acid, which reacts with phenylhydrazine hydrochloride to form phenylhydrazone glyoxalate. The substance has an absorption peak at 324 nm, and its OD value is proportional to the concentration of phenylhydrazone glyoxalate in a certain range, and the amount of phenylhydrazone generated reflects the activity of glycolate oxidase.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Extraction Solution	60 mL × 1 vials	60 mL × 2 vials	2-8℃, 12 months
Reagent 2	Buffer Solution	45 mL × 1 vials	45 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 3	Chromogenic Agent	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months, shading light
Reagent 4	Substrate	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Micropipettor, Vortex mixer, Incubator, Centrifuge, Spectrophotometer(324 nm），Optical path cuvette (1 mL of volume, 1 cm optical diameter)

Reagents:

Double distilled water

Reagent preparation

Size 1(50 Assays)：

① Keep extraction solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of working solution：
Dilute one vial of chromogenic agent with 12 mL of purified water. Aliquoted storage at -20°C protected from light, and avoid repeated freeze/thaw cycles is advised.

Size 2(100 Assays)：

① Keep extraction solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of working solution：
Dilute one vial of chromogenic agent with 24 mL of purified water. Aliquoted storage at -20°C protected from light, and avoid repeated freeze/thaw cycles is advised.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 12000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the supernatant sample can be stored at -20℃ for 2 days.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Oxalis corniculata tissue homogenate	1
10% Ginkgo biloba tissue homogenate	1
10% Cactus tissue homogenate	1
10% Bamboo leaf tissue homogenate	1
10% Osmanthus fragrans leaf tissue homogenate	4-8
10% Camphor trees leaf tissue homogenate	3-5

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① When the OD value is more than 1.0, the sample must be diluted.

② If use a mortar to prepare tissue homogenate, precool the mortar at a 4°C environment.

Operating steps

① Blank tubes: Take 50 μL of double distilled water to 5 mL EP tube.
Sample tubes: Take 50 μL of sample to 5 mL EP tube.

② Add 650 μL of buffer solution, 200 μL of working solution and 100 μL of substrate to each tube.

③ Mix fully and set to zero with double distilled water and measure the OD value of each tube with 1 mL of volume and 1 cm optical path cuvette at 324 nm, recorded as A_1.

④ Stand for 3 min and detect again, recorded as A_2.

Calculation

The sample:

Tissue sample:

Definition: the amount of enzyme in 1 mg of tissue protein that oxidize 1 nmol glycollic acid at room temperature for 1 min is defined as 1 activity unit.

Glycolate oxidase activity (U/mgprot) = $\Delta A \div (\varepsilon \times d) \div 50 \times 1000 \times f \times 10^3 \div T \div C_{pr}$

[Note]

ΔA: $\Delta A = (A_2 - A_1) - (A_{02} - A_{01})$.

A_1: The OD value of initial sample tube.

A_2: The OD value of sample tube after 3 min.

A_{01}: The OD value of initial blank tube.

A_{02}: The OD value of blank tube after 3 min.

ε: The molar extinction coefficient of phenylhydrazone glyoxylate, 17 L/(mmol•cm).

d: The optical path of cuvette, 1 cm.

50: The volume of sample added to the reaction, 50 μL.

1000: The total volume of reaction, 1000 μL.

f: The dilution multiple of tested samples.

T: Reaction time, 3 min.

Cpr: The concentration of protein in sample, mgprot/mL.

10^3: 1 μmol = 1000 nmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three cactus tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	5.80	85.50	179.00
%CV	1.3	1.2	0.8

Inter-assay Precision

Three cactus tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	5.80	85.50	179.00
%CV	8.0	8.1	8.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	26.4	94.5	156.2
Observed Conc. (U/mL)	26.1	100.2	157.8
Recovery rate (%)	99	106	101

Sensitivity

The analytical sensitivity of the assay is 0.3 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

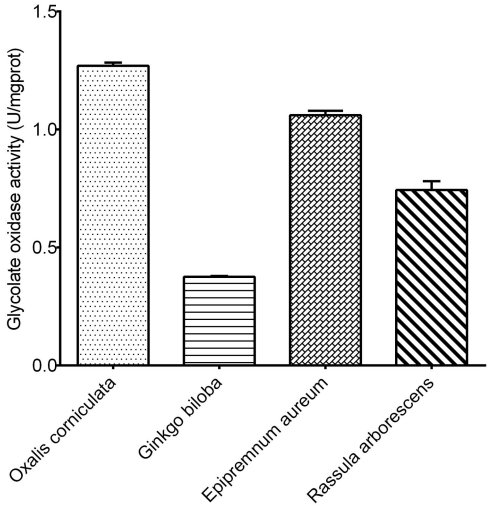
Example analysis：

For oxalis corniculata, take 50 μL tissue supernatant and carry the assay according to the operation table. The results are as follows:

The A_{01} of the blank is 0.008, the A_{02} of the blank is 0.010, the A_1 of the sample is 0.577, the A_2 of the sample is 0.608, $\Delta A = (0.608 - 0.577) - (0.010 - 0.008) = 0.029$, the concentration of protein in sample is 8.93 mgprot/mL, and the calculation result is:

Glycolate oxidase activity (U/mgprot) = $0.029 \div (17 \times 1) \div 50 \times 1000 \times 10^3 \div 3 \div 8.93 = 1.27$ U/mgprot

Detect 10% oxalis corniculata tissue homogenate(the concentration of protein is 8.93 mgprot/mL), 10% ginkgo blioba tissue homogenate(the concentration of protein is 3.04 mgprot/mL), 10% epipremnum aureum tissue homogenate (the concentration of protein is 5.39 mgprot/mL) and 10% rassula arborescens tissue homogenate (the concentration of protein is 5.97 mgprot/mL) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.