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

Catalog No：E-BC-K259-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader(410 nm)

Elabscience® Polyphenol Oxidase (PPO) 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

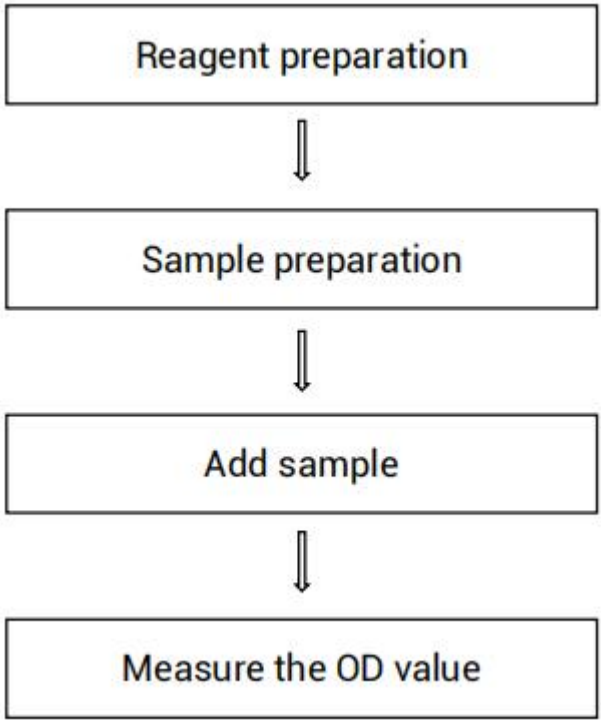
Email: techsupport@elabscience.com

Website: www.elabscience.com

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

Table of contents

Assay summary



Intended use

This kit can be used to measure polyphenol oxidase (PPO) activity in plant tissue samples.

Detection principle

Polyphenol oxidase (PPO) can catalyze phenolic compounds into quinone substances. The latter has specific absorption at 410 nm. The activity of PPO can be calculated indirectly bymeasuring the OD value at 410 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Buffer Solution	40 mL × 1 vial	40 mL × 2 vials	2-8°C, 12 months
Reagent 3	Substrate	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Microplate reader (410 nm), Tubes, Micropipette, Vortex mixer, 100°C Water bath

Reagents:

Double distilled water

Reagent preparation

① Preheat extracting solution in a water bath (37°C) and until the extracting solution looks clear.

② Equilibrate buffer solution and substrate to room temperature before use.

Sample preparation

① Sample preparation

Extraction of crude enzyme solution A

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

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4°C.

④ Centrifuge at 11000×g for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Extraction of crude enzyme solution B (For control tubes)

After the crude enzyme solution A was extracted, 50% of the supernatant was taken to a new 1.5 mL EP tube and boiled at 100°C for 5 min. Cool the tubes with running water and crude enzyme solution B was prepared

② Dilution of sample

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

Sample type	Dilution factor
10% Ginger tissue homogenization	1
10% Chinese yam tissue homogenization	1
10% Corn tissue homogenization	1
10% Pear tissue homogenization	1

Note: For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The temperature and time of incubation at 37°C must be accurately.
② The explosion-proof EP tubes are recommended to use for the 100°C water bath.
③ It is a normal phenomenon that suspended substance appeared in some tubes, you can centrifuge at 11000×g for 15 min at room temperature, then take the supernatant for measuring the OD value.

Operating steps

① Control tube: Add 600 μL buffer solution into 1.5 mL EP tubes.
Sample tube: Add 600 μL buffer solution into 1.5 mL EP tubes.
② Add 150 μL substrate into each tubes.
③ Control tube: Add 150 μL of crude enzyme solution B into control tubes.
Sample tube: Add 150 μL of crude enzyme solution A into sample tubes.
④ Mix well with the vortex mixer, incubate accurately at 37°C for 3 min, incubate at 100°C water bath for 5 min immediately. Then cool the tubes to room temperature with running water.
⑤ Take 320 μL into the microplate and measure the OD value of each well at 410 nm (the OD value of the sample well is record as A_1, the OD value of the control well is record as A_2, $\triangle A = A_1 - A_2$).

Calculation

Definition: 0.01 OD value changed at 410 nm by 1 mg of tissue protein sample per minute in the reaction system at 37°C that is defined as an enzyme activity unit.

The sample:

$$\text{PPO activity (U/mgprot)} = \frac{\Delta A}{0.01} \div V \div Cpr \div T \times f = 222.2 \times \Delta A \div Cpr \times f$$

[Note]

ΔA: $\triangle A = A_1 - A_2$

V: The volume of sample added to the reaction, 0.15 mL.

T: Reaction time, 3 min;

C_{pr}: The concentration of protein in sample, mgprot/mL.

f: The dilution factor of sample before tested.

Appendix I Example Analysis

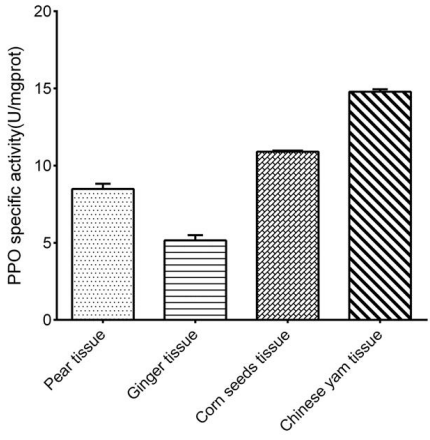
Example analysis：

For chinese yam tissue, take 0.1 g of chinese yam tissue, add 0.9 mL of extracting solution, then homogenize the sample in ice water bath, centrifuge at 10000 g for 10 min at 4°C, then take 0.15 mL of chinese yam tissue supernatant and carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample (A_1) is 0.320, the average OD value of the control (A_2) is 0.189, $\triangle A = A_1 - A_2 = 0.320 - 0.189 = 0.131$, the concentration of protein in sample is 1.97 mgprot/mL, and the calculation result is:

$$\text{PPO activity (U/mgprot)} = 222.2 \times 0.131 \div 1.97 = 14.78 \text{ U/mgprot}$$

Detect 10% pear tissue tissue homogenate (the concentration of protein is 2.75 mgprot/mL), 10% ginger tissue homogenate (the concentration of protein is 2.26 mgprot/mL), 10% corn seeds tissue homogenate (the concentration of protein is 2.58 mgprot/mL), 10% Chinese yam tissue homogenate (the concentration of protein is 1.97 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.