Technical Manual

Polyphenol Oxidase (PPO) Activity Assay Kit

- Catalogue Code: MAES0163
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Polyphenol oxidase (PPO) is one of the most widely distributed metalloproteinases in the nature. It is ubiquitous in plants, fungi, and insects. The activity of polyphenol oxidase can be detected even on the decaying plant residues of the soil. PPO catalyzes the formation of lignin and quinone compounds, which can prevent cells from being harmed by pathogens, and can also play a direct role in disease resistance by forming quinone substances. Therefore, through the research on the activity of PPO, it can more directly reflect the disease resistance of plant organism in the process of growth.

3. Intended Use

This kit can be used to detect Polyphenol Oxidase (PPO) activity in plant tissue samples.

4. 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.

5. Kit components & storage

Item	Specification	Storage
Extracting solution	60 mL × 2 vials	2-8°C, 6 months
Buffer Solution	40 mL × 2 vials	2-8°C, 6 months
Substrate	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (410 nm)
- Spectrophotometer (240 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. The temperature and time of incubation at 37°C must be accurate.

2. Explosion-proof EP tubes are recommended for the 100°C water bath.

3. If suspended substances appear in some tubes, you can centrifuge at 11000 g for 15 min at room temperature, then take the supernatant for measuring the OD value.

7. Reagent preparation:

1. Preheat the extracting solution at 37°C for 20 min before use, use it once it has been completely clarified.

2. Bring the buffer solution and substrate to room temperature before use.

8. Sample Preparation

Tissue sample:

Extraction of crude enzyme solution A

Accurately weigh the plant tissue sample, add extracting solution according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 11000 g for 15 min, take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (MAES0177).

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 1.5mL EP tube and boiled at 100°C for 5 min. Cool the tubes with running water and crude enzyme solution B was prepared.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment.

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

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 410 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1′	S9	S9′	S17	S17′	S25	S25′	S33	S33′	S41	S41′
B	S2	S2′	S10	S10′	S18	S18′	S26	S26′	S34	S34′	S42	S42′
C	S3	S3′	S11	S11′	S19	S19′	S27	S27′	S35	S35′	S43	S43′
D	S4	S4′	S12	S12′	S20	S20′	S28	S28′	S36	S36′	S44	S44′
E	S5	S5′	S13	S13′	S21	S21′	S29	S29′	S37	S37′	S45	S45′
F	S6	S6′	S14	S14′	S22	S22′	S30	S30′	S38	S38′	S46	S46′
G	S7	S7′	S15	S15′	S23	S23′	S31	S31′	S39	S39′	S47	S47′
H	S8	S8′	S16	S16′	S24	S24′	S32	S32′	S40	S40′	S48	S48′

Note: S1-S48, sample wells; S1′-S48′, control wells.

10. Operation Steps

1. **Sample tube:** Add 600 μL buffer solution into 1.5 mL EP tubes.
   Control tube: Add 600 μL buffer solution into 1.5 mL EP tubes.

2. Add 150 μL substrate into each tube.

3. **Sample tube:** Add 150 μL of crude enzyme solution A into sample tubes.
   Control tube: Add 150 μL of crude enzyme solution B into control tubes.

4. Mix fully 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, $\triangle A = A_1 - A_2$).

Operation Table

	Sample tube	Control tube
Buffer solution (μL)	600	600
Substrate (μL)	150	150
Crude enzyme solution A (μL)	150	
Crude enzyme solution B (μL)		150
Mx fully 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. 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, $\triangle A = A_1 - A_2$).		

11. Calculations

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.

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

ΔA: 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 test

12. Performance Characteristics

Average inter-assay CV (%)	8.7
Average intra-assay CV (%)	4.6

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, carry the assay according to the operation table.

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:

PPO activity (U/mgprot) = 222.2 × 0.131 ÷ 1.97 = 14.78 U/mgprot

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com