# TECHNICAL MANUAL

# Polyphenol Oxidase (PPO) Activity Assay Kit

- **SKU CODE:** MAES0163
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Assay summary

- Reagent preparation
- Sample preparation
- Operating steps
- Calculation

# 2. Intended use

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

# 3. Detection principle

Polyphenol oxidase (PPO) catalyzes the conversion of phenolic compounds into quinone substances. These quinones exhibit specific absorption at 410 nm. The activity of PPO can be calculated indirectly by measuring the optical density (OD) value at 410 nm.

# 4. 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, protected from light |
| | Microplate | 48 wells | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | 2 pieces | |

# 5. Materials prepared by users

**Instruments:**

Microplate reader (410 nm), tubes, micropipette, vortex mixer, 100 °C water bath

**Reagents:**

Double-distilled water

# 6. Reagent preparation

1. Preheat the extracting solution in a water bath at 37 °C until the solution appears clear.
2. Equilibrate the buffer solution and substrate to room temperature before use.

# 7. Sample preparation

Sample preparation

Extraction of crude enzyme solution A

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

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

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

4. Centrifuge at 11,000×g for 15 minutes to remove insoluble material. Collect the supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of the supernatant (MAES0126, MAES0402).

Extraction of crude enzyme solution B (For control tubes)

After extracting crude enzyme solution A, take 50% of the supernatant to a new 1.5 mL EP tube and boil at 100 °C for 5 minutes. Cool the tubes with running water to prepare crude enzyme solution B.

Dilution of sample

The recommended dilution factors for different samples are as follows (for reference only):

10% Ginger tissue homogenization: 1

10% Chinese yam tissue homogenization: 1

10% Corn tissue homogenization: 1

10% Pear tissue homogenization: 1

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

# 8. The key points of the assay

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. It is normal for suspended substances to appear in some tubes. You can centrifuge at 11,000×g for 15 minutes at room temperature, then use the supernatant for measuring the OD value.

# 9. 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 tube.
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 minutes, then immediately incubate in a 100 °C water bath for 5 minutes. Cool the tubes to room temperature with running water.
5. Transfer 320 μL into the microplate and measure the OD value of each well at 410 nm (record the OD value of the sample well as A1, the OD value of the control well as A2, ΔA = A1 - A2).

# 10. Calculation

**Definition:** One enzyme activity unit is defined as the amount of enzyme that causes a 0.01 OD value change at 410 nm per minute per mg of tissue protein in the reaction system at 37 °C.

For the sample:

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

[Note]

ΔA: $\Delta A = A_1 - A_2$

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

T: Reaction time, 3 minutes

$C_{pr}$: The concentration of protein in the sample, $mg_{prot}/mL$

f: The dilution factor of the sample before testing

# 11. 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 an ice water bath. Centrifuge at 10,000×g for 10 minutes at 4 °C, then take 0.15 mL of Chinese yam tissue supernatant and perform the assay according to the operating steps.

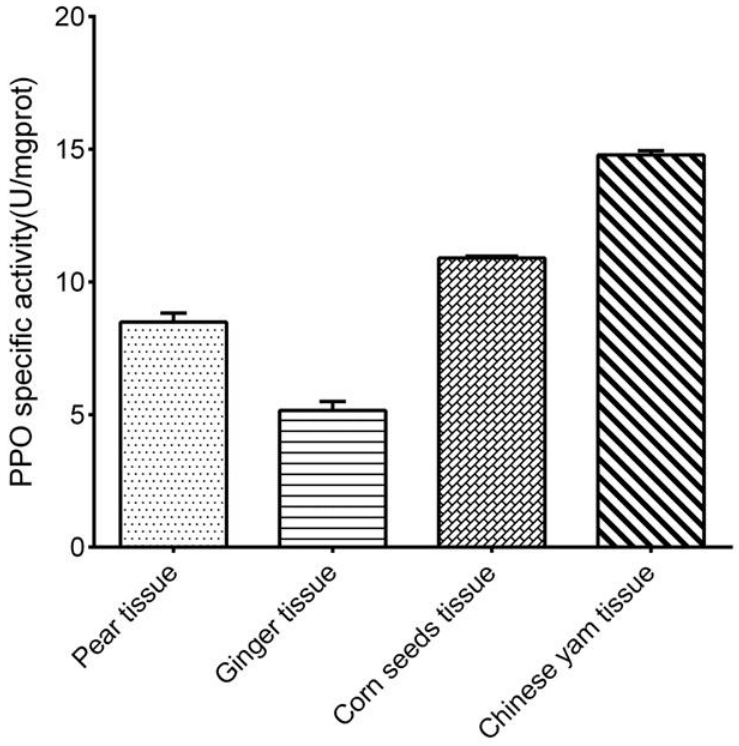
The results are as follows:

The average OD value of the sample ($A_1$) is 0.320, the average OD value of the control ($A_2$) is 0.189, $\Delta A = A_1 - A_2 = 0.320 - 0.189 = 0.131$. The concentration of protein in the sample is 1.97 $mg_{prot}$/mL, and the calculation result is:

PPO activity (U/$mg_{prot}$) = 222.2 × 0.131 ÷ 1.97 = 14.78 U/$mg_{prot}$

Detection results for various tissue homogenates according to the protocol:

- 10% pear tissue homogenate (protein concentration: 2.75 $mg_{prot}$/mL)
- 10% ginger tissue homogenate (protein concentration: 2.26 $mg_{prot}$/mL)
- 10% corn seeds tissue homogenate (protein concentration: 2.58 $mg_{prot}$/mL)
- 10% Chinese yam tissue homogenate (protein concentration: 1.97 $mg_{prot}$/mL)




# 12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**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.

**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**