TECHNICAL MANUAL

Peroxidase (POD) Activity Assay Kit (Plant samples)

- **SKU CODE:** MAES0152
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- ![img-0.jpeg](img-0.jpeg)

2. Intended use

This kit can be used to measure the peroxidase (POD) activity in plant tissue samples, but not for serum (plasma).

3. Detection principle

The peroxidase can catalyze the decomposition of H_{2O}_2 and produce water and oxygen. Oxygen oxidizes pyrogallic acid to form a yellow product. The activity of peroxidase can be calculated by measuring the absorbance at 420 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Substrate Solution	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
Reagent 4	Stop Solution	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (410-430 nm), Vortex mixer, Centrifuge, 37°C Incubator

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. **Preparation of chromogenic application solution::** Dilute one vial of chromogenic agent with 8.75 mL of double distilled water, mix well. Store at 2-8°C for 1 month protected from light.
2. **Preparation of substrate application solution::** For each well, prepare 110 µL of substrate application solution (mix well 4.4 µL of substrate solution and 105.6 µL of double distilled water). The substrate application solution should be prepared fresh. Store at 2-8°C for 7 days.
3. **Preparation of stop application solution::** For each well, prepare 200 µL of stop application solution (mix well 100 µL of stop solution and 100 µL of double distilled water). The stop application solution should be prepared fresh. Store at 2-8°C for 7 days.

7. Sample preparation

Tissue sample preparation:

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

2. Wash tissue in cold double distilled water.

3. Homogenize 20 mg tissue in 180 µL PBS (0.01 M, pH 7.4) with a Dounce homogenizer at 4°C.

4. Centrifuge at 10,000 xg for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Dilution of sample:

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

- 10% Green pepper tissue homogenate: 1

- 10% Chive leaf tissue homogenate: 1

- 10% Photinia leaf tissue homogenate: 1

- 10% Epipremnum aureum tissue homogenate: 1

- 10% Mushrooms tissue homogenate: 1

- 10% White radish tissue homogenate: 1

Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The reaction time must be controlled strictly.

2. Light should be prevented during the experiment to avoid large differences between multiple wells.

3. Do not take precipitate when collecting the supernatant for measuring the OD value to avoid the effect of precipitate on OD value.

4. The step of measuring the OD value must be finished within 30 minutes.

5. If the OD value of sample tube is more than 0.7, the sample must be diluted and tested again.

9. Operating steps

1. **Sample tube::** Add 380 μL of buffer solution, 90 μL of chromogenic application solution, 110 μL of substrate application solution and 20 μL of sample into a 1.5 mL EP tube. Control tube: Add 380 μL of buffer solution, 90 μL of chromogenic application solution, 110 μL of double distilled water and 20 μL of sample into a 1.5 mL EP tube.
2. Mix fully with the vortex mixer, then incubate at 37°C for 30 minutes accurately.
3. Add 200 μL of stop application solution into each tube, mix well and centrifuge at 2300 xg for 10 minutes.
4. Take 300 μL of supernatant from each tube to the corresponding wells. Measure the OD values of each well at 420 nm with microplate reader.

10. Calculation

Definition: The enzyme amount that catalyzes 1 μg substrate by 1 mg tissue protein per minute at 37°C is defined as 1 unit.

POD activity (U/mg_{prot}) = (ΔA ÷ 12*) × 1 × V_1 ÷ V_2 ÷ t ÷ (C_{pr} ÷ f) × 1000*

[Note]

ΔA: $OD_{Sample} - OD_{Control}$

1: the optical diameter with volume of 300 μL added to the microplate, 1 cm

V_1: the total volume of the reaction, 800 μL

V_2: the volume of sample added to the reaction, 20 μL

t: reaction time, 30 minutes

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

f: dilution factor of sample before tested

1000*: 1000 μg = 1 mg

12*: absorption coefficient

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	5.80	36.20	75.00
%CV	3.6	3.1	2.9

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	5.80	36.20	75.00
%CV	3.6	3.1	8.3

Recovery

Three samples of high concentration, middle concentration and low concentration were tested 6 times in parallel to obtain an average recovery rate of 102%.

Recovery

Sample 1	Sample 2	Sample 3
12.5	47.6	88.5
12.4	50.0	90.3
99	105	102

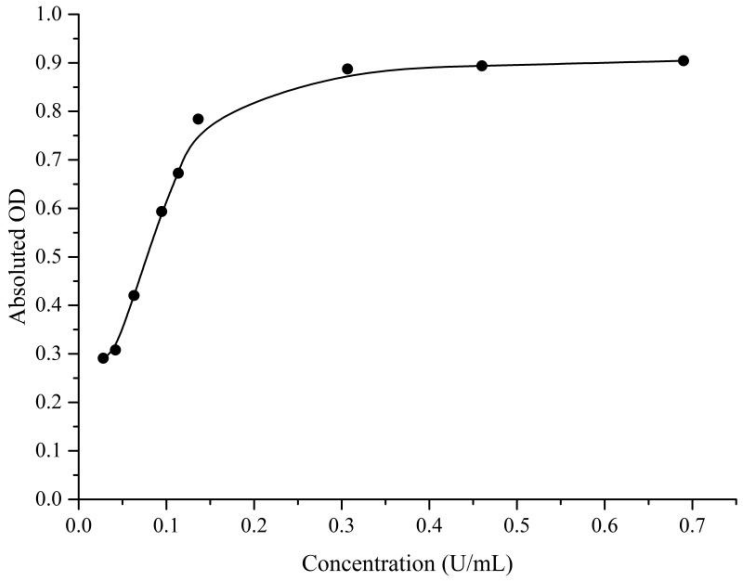
Sensitivity

The analytical sensitivity of the assay is 0.01 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.

Standard curve

Note: It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only.

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



12. Appendix II Example Analysis

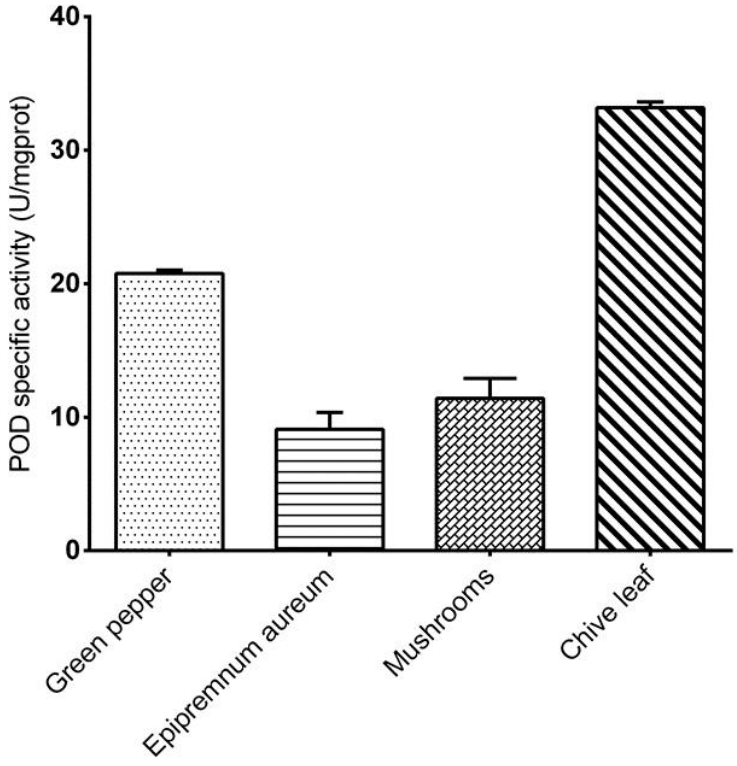
Example analysis:

Take 20 µL of 10% green pepper tissue homogenate supernatant and carry out the assay according to the operation steps. The results are as follows:

The OD value of the sample is 0.578, the OD value of the control is 0.171, the absolute OD value is 0.406, the concentration of protein in sample is 2.18 mg_{prot}/mL, and the calculation result is:

POD activity (U/mg_{prot}) = 0.406 ÷ (12 × 1) × 0.8 ÷ 0.02 ÷ 30 ÷ (2.18 ÷ 1) × 1000 = 20.69 U/mg_{prot}

Detect 10% green pepper tissue homogenate (the concentration of protein is 2.18 mg_{prot}/mL), 10% epipremnum aureum tissue homogenate (the concentration of protein is 1.21 mg_{prot}/mL), 10% mushrooms tissue homogenate (the concentration of protein is 1.33 mg_{prot}/mL) and 10% chive leaf tissue homogenate (the concentration of protein is 2.12 mg_{prot}/mL) according to the protocol, the result is as follows:



13. 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 pre-test 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.