Technical Manual

Peroxidase (POD) Colorimetric Assay Kit (Plant samples)

- Catalogue Code: MAES0152
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.01-100 U/mL

Sensitivity:

0.01 U/mL

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

Plant peroxidase, a member of the superfamily of peroxidase, catalyzes the redox reaction between H_2O_2 and various reductants. The plant peroxidase has the same general structure and consists of iron porphyrin IX and ten α-helixes. Based on the difference of primary structure, the superfamily of plant peroxidase can be divided into three types: class I (intracellular type), class II (extracellular type of fungi) and class III (secreted type of plant).

3. Intended Use

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

4. Detection Principle

The peroxidase can catalyze the decomposition of H_2O_2 and produce water and oxygen. And oxygen oxidized pyrogallic acid to form yellow product. The activity of peroxidase can be calculated by measuring the absorbance at 420 nm.

5. Kit components & storage

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

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (410-430 nm)
- Spectrophotometer (240 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. The reaction time must be strictly controlled.

2. Avoid taking the precipitate when collecting the supernatant.

3. Measure the OD value in 30 min.

4. During detection, the cuvettes should be washed.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. The preparation of **chromogenic agent application solution:** Dissolve a vial of chromogenic agent with 8.75 mL double distilled water fully. The prepared solution can be store at 2-8°C with avoid direct sunlight for 1 month.

3. The preparation of **substrate application solution:** Dilute the substrate solution with double distilled water for 25 times. The OD should be about 0.395-0.405 when set spectrophotometer to zero with double distilled water at 240 nm with 1 cm optical path cuvette. If the OD value is too high, then dilute with double-distilled water. While the OD value is too low, add appropriate substrate solution. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 7 days.

4. Preparation of **stop application solution:** Mix the stop solution and double distilled water at a ratio of 1:1. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 7 days.

8. Sample Preparation

Tissue sample:
Weigh 0.020-1 g fresh plant tissue and wash with double distilled water, absorb moisture on the surface of tissue with filter paper. Then add PBS (0.01 M, pH 7.4) according to the ratio of the volume of PBS (mL): the weight of the tissue (g) =9:1. Homogenize the sample on ice and centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (MAES0177).

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 and the detection range (0.01-100 U/mL).

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

Sample Type:	Dilution Factor
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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 420 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 380 μL of buffer solution, 90 μL of chromogenic agent 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 agent application solution, 110 μL of double-distilled water and 20 μL of sample into a 1.5 mL EP tube.

2. Oscillate fully with the vortex mixer, then incubate at 37°C for 30 min accurately.

3. Add 200 μL of stop application solution into each tube, mix fully and centrifuge at 2300 g for 10 min.

4. Take 300 μL of supernatant of each tube to the corresponding wells. Measure the OD values of each well at 420 nm with microplate reader.

Operation Table

	Control tube	Sample tube
Buffer solution (μL)	380	380
Chromogenic agent application solution (μL)	90	90
Substrate application solution (μL)		110
Double-distilled water (μL)	110	
Sample (μL)	20	20
Oscillate fully with the vortex mixer, then incubate at 37°C for 30 min accurately.		
Stop application solution (μL)	200	200
Mix fully and centrifuge at 2300 g for 10 min. Take 300 μL of supernatant of each tube to the corresponding wells. Measure the OD values of each well at 420 nm with microplate reader		

11. Calculations

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

$$\text{POD activity (U/mgprot)} = \frac{\Delta A}{12^{*} \times 1} \times \frac{V_1}{V_2} \div t \div (C_{pr} \div f) \times 1000^{*}$$

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 min
C_{pr}: concentration of protein in sample, mgprot/mL
f: dilution factor of sample before test
1000*: 1000 μg=1 mg
12*: absorption coefficient

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12. Performance Characteristics

Detection Range	0.01-100 U/mL
Sensitivity	0.01 U/mL
Average recovery rate (%)	102
Average inter-assay CV (%)	5.0
Average intra-assay CV (%)	3.2

Analysis

Take 20 μL of 10% green pepper tissue homogenate supernatant, carry the assay according to the operation table.

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 mgprot/mL, and the calculation result is:

POD activity (U/mgprot)

= 0.406 ÷ (12×1) × 0.8 ÷ 0.02 ÷ 30 ÷ (2.18÷1) × 1000 = 20.69 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.

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