PRODUCT INFORMATION & MANUAL

Peroxidase Activity Assay Kit (Colorimetric)

NBP3-24529

For research use only.
Not for diagnostic or therapeutic procedures.

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Peroxidase (POD) Activity Assay Kit
(Plant Samples)

Catalog No: NBP3-24529

Method: Colorimetric method

Specification: 100 Assays (Can detect 50 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.5 U/mL

Detection range: 0.5-40 U/mL

Average intra-assay CV (%): 3.8

Average inter-assay CV (%): 5.5

Average recovery rate (%): 98

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure the Peroxidase (POD) activity in plant tissue samples.

▲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).

▲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.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 4 vials	2-8℃ , 12 months
Reagent 2	Chromogenic Agent	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	Substrate Solution	1.5 mL × 4 vials	2-8℃ , 12 months
Reagent 4	Stop Solution	60 mL × 1 vial	2-8℃ , 12 months
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.			

▲Materials prepared by users

Instruments

Spectrophotometer (240 nm & 420nm), Incubator, Vortex mixer, Micropipettor, Centrifuge

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key points of the assay

1. The reaction time must be controlled strictly.
2. The light should be prevented during the experiment, so as to avoid the phenomenon that the difference between the multiple wells is too large.
3. The step of measuring the OD value must be finished in 30 min.
4. If the OD value of sample tube is more than 0.6, the sample must be diluted and test again.
5. During the detection, the cuvettes should be washed, so as to avoid the residual water in the cuvette to affect the results.

Pre-assay preparation

▲Reagent preparation

1. The preparation of chromogenic agent application solution:
   Dissolve a vial of reagent 2 with 17.5 mL double distilled water fully. The prepared solution can be store at 2-8℃ with shading light.
2. The preparation of reagent 3 application solution:
   Dilute the reagent 3 with double distilled water for 25 times. The OD should be about 0.395-0.405 (optical path=1 cm) when set spectrophotometer to zero with double-distilled water at 240 nm. If the OD value is too high, then dilute with double-distilled water. While the A value is too low, add appropriate Reagent 3 (The dilution factor is about 25 in general).
3. Preparation of Reagent 4 application solution:
   Dilute the reagent 4 with double distilled water at a ratio of 1:1 before use.

▲Sample preparation

Plant tissue

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M, E-BC-K168-S).

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.5-40 U/mL).

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

Sample type	Dilution factor
10% Chinese rose tissue homogenization	1
10% Epipremnum aureum tissue homogenization	2-3
10% Green pepper tissue homogenization	1
10% Mushrooms tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Detailed operating steps

(1) Sample tube: Add 2.4 mL of reagent 1, 0.3 mL of chromogenic agent application solution, 0.2 mL of reagent 3 application solution and 0.1 mL of sample into a 5 mL EP tube.
Control tube: Add 2.4 mL of reagent 1, 0.3 mL of chromogenic agent application solution, 0.2 mL of double distilled water and 0.1 mL of sample into a 5 mL EP tube.

(2) Oscillate fully with the vortex mixer, then incubate the tubes at 37℃ for 30 min accurately.

(3) Add 1.0 mL of reagent 4 application solution into each tube, mix fully and centrifuge at 2300 g for 10 min. Take the supernatant for detect the OD value.

(4) Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 420 nm with 1 cm optical path cuvette (This step must be finished in 30 min).

▲Summary operation table

	Sample tube	Control tube
Reagent 1 (mL)	2.4	2.4
Chromogenic agent application solution (mL)	0.3	0.3
Reagent 3 application solution (mL)	0.2	
Double distilled water (mL)		0.2
Sample (mL)	0.1	0.1
Incubate at 37℃ for 30 min accurately.		
Reagent 4 application solution (mL)	1.0	1.0
Mix fully and centrifuge for 10 min. Take the supernatant. Set the spectrophotometer to zero with double distilled water and measure the OD values at 420 nm.		

▲Calculation

Definition:

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

Calculation formula:

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

Note:

ΔA: OD_{Sample}-$OD_{Control}$

1: The optical path of cuvette, 1 cm

V_1: The total volume of reaction solution, 4 mL

V_2: The volume of sample added to reaction system, 0.1 mL

t: Reaction time, 30 min

C_{pr}: Concentration of protein in sample (mgprot/mL)

f: Dilution factor of sample before test.

*: Constant

Appendix I Data

▲Example analysis

For daucus carota tissue, take 0.1 mL of daucus carota tissue supernatant, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample tube is 0.263, the average OD value of the control tube is 0.088, the concentration of protein in sample is 0.77 mg/mL, and the calculation result is:

$$\text{POD activity (U/mgprot)} = \frac{0.263-0.088}{12\times1} \times \frac{3}{0.1} \div 30 \div 0.77 \times 1000 \times 1$$
$$= 18.94 \text{ U/mgprot}$$

Appendix II References

1. Hiraga S, Sasaki K, Ito H, et al. A large family of class III plant peroxidases [J]. Plant & Cell Physiology, 2001, 42(5): 462-468.
2. Jouili H, Bouazizi H, Ferjani E E. Plant peroxidases: biomarkers of metallic stress[J]. Acta Physiologiae Plantarum, 2011, 33(6): 2075-2082.
3. Passardi F, Penel C, Dunand C. Performing the paradoxical: how plant peroxidases modify the cell wall[J]. Trends in Plant Science, 2004, 9(11): 534-540.
4. Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a Swiss army knife[J]. Plant Cell Reports, 2005, 24(5): 255-265.