PRODUCT INFORMATION & MANUAL

Peroxidase Activity Assay Kit (Colorimetric)

NBP3-24528

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

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Peroxidase (POD) Activity Assay Kit (Plant Samples)

Catalog No: NBP3-24528

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.01 U/mL

Detection range: 0.01-100 U/mL

Average intra-assay CV (%): 3.2

Average inter-assay CV (%): 5.0

Average recovery rate (%): 102

- ▲ 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, but not for serum (plasma).

▲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 × 1 vial	2-8℃ , 12 months
Reagent 2	Chromogenic Agent	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	Substrate Solution	1 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Stop Solution	20 mL × 1 vial	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader (410-430 nm), Micropipettor, Vortex mixer, Centrifuge, 37℃ Incubator

Reagents

Double distilled water, 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. Don't take the precipitate when take the supernatant for measuring the OD value to avoid the effect of precipitate to OD value.
4. The step of measuring the OD value must be finished in 30 min.
5. If the OD value of sample tube is more than 0.7, the sample must be diluted and test again.

Pre-assay preparation

▲Reagent preparation

1. The preparation of reagent 2 application solution:
   Dissolve a vial of reagent 2 with 8.75 mL double distilled water fully. The prepared solution can be stored at 2-8℃ with shading light for 1 month.
2. The preparation of reagent 3 application solution:
   Dilute the reagent 3 with double distilled water at a ratio of 1:24 before use and the prepared solution can be stored at 2-8℃ for 7 days.
3. Preparation of Reagent 4 application solution:

   Dilute the reagent 4 with double distilled water at a ratio of 1:1 before use and the prepared solution can be stored at 2-8℃ for 7 days.

▲Sample preparation

Plant tissue

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℃ . Take the supernatant and 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 ((0.01-100 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 PBS (0.01 M, pH 7.4).

Assay protocol

▲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.

▲Detailed operating steps

(1) Sample tube: Add 380 μL of reagent 1, 90 μL of reagent 2 application solution, 110 μL of reagent 3 application solution and 20 μL of sample into a 1.5 mL EP tube.

Control tube: Add 380 μL of reagent 1, 90 μL of reagent 2 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 the tubes at 37℃ for 30 min accurately.

(3) Add 200 μL of reagent 4 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.

▲Summary operation table

	Control tube	Sample tube
Reagent 1 (μL)	380	380
Reagent 2 application solution (μL)	90	90
Reagent 3 application solution (μL)		110
Double distilled water (μL)	110	
Sample (μL)	20	20
Oscillate fully, then incubate at 37℃ for 30 min accurately.		
Reagent 4 application solution (μL)	200	200
Mix fully and centrifuge for 10 min. Take 300 μL of supernatant of each tube to the corresponding wells. Measure the OD values ofl 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 diameter with volume of 300 μl added to the microplate, 1 cm

V_1: The total volume of reaction solution, 800 μL

V_2: The volume of sample added to reaction system, 20 μL

t: Reaction time, 30 min

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

f: Dilution factor of sample before test.

12*: absorption coefficient

1000*: 1000 μg=1 mg.

Appendix I Data

▲Example 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 concentration of protein in sample is 2.18 mgprot/mL, and the calculation result is:

$$\text{POD activity (U/mgprot)}=\frac{0.578-0.171}{12\times1}\times0.8\div0.02\div30\div(2.18\div1)\times1000$$
$$=20.69\ \text{U/mgprot}$$

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.