PRODUCT INFORMATION & MANUAL

Polyphenol Oxidase/PPO Activity Assay Kit (Colorimetric)

NBP3-25778

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

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Polyphenol Oxidase (PPO) Activity Assay Kit

Catalog No: NBP3-25778

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Average intra-assay CV (%): 4.6

Average inter-assay CV (%): 8.7

- ▲ 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 detect Polyphenol Oxidase (PPO) activity in plant tissue samples.

▲Background

Polyphenol oxidase (PPO) is one of the most widely distributed metalloproteinases in the nature. It is ubiquitous in plants, fungi, and insects. The activity of polyphenol oxidase can be detected even on the decaying plant residues of the soil. PPO catalyzes the formation of lignin and quinone compounds, which can prevent cells from being harmed by pathogens, and can also play a direct role in disease resistance by forming quinone substances. Therefore, through the research on the activity of PPO, it can more directly reflect the disease resistance of plant organism in the process of growth.

▲Detection principle

Polyphenol oxidase (PPO) can catalyze phenolic compounds into quinone substances. The latter has specific absorption at 410 nm. The activity of PPO can be calculated indirectly bymeasuring the OD value at 410 nm.

▲Kit components & Storage

	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Buffer Solution	40 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Substrate	20 mL × 1 vial	2-8℃ , 12 months, shading light
	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.			

▲Materials prepared by users

Instruments

Microplate reader (410 nm), Tubes, Micropipette, Vortex mixer, 100℃ Water bath

Reagents:

Double distilled water

⚠ 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 temperature and time of incubation at 37℃ must be accurately.
2. The explosion-proof EP tubes are recommended to use for the 100℃ water bath.
3. It is a normal phenomenon that suspended substance appeared in some tubes, you can centrifuge at 11000 g for 15 min at room temperature, then take the supernatant for measuring the OD value.

Pre-assay preparation

▲Reagent preparation

1. Preheat the reagent 1 at 37℃ for 20 min before use, and then use it after completely clarified.
2. Bring the reagent 2 and reagent 3 to room temperature before use.

▲Sample preparation

1. Extraction of crude enzyme solution A

Accurately weigh the plant tissue sample, add reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 11000 g for 15 min, take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M, E-BC-K168-S).

2. Extraction of crude enzyme solution B (For control tubes)

After the crude enzyme solution A was extracted, 50% of the supernatant was taken to a new 1.5mL EP tube and boiled at 100℃ for 5 min. Cool the tubes with running water and crude enzyme solution B was prepared.

▲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.

Sample type	Dilution factor
10% Ginger tissue homogenization	1
10% Chinese yam tissue homogenization	1
10% Corn tissue homogenization	1
10% Pear tissue homogenization	1

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

The measurement of samples

1. Control tube: Add 600 μL reagent 2 into 1.5 mL EP tubes.
   Sample tube: Add 600 μL reagent 2 into 1.5 mL EP tubes.
2. Add 150 μL reagent 3 into each tubes.
3. Sample tube: Add 150 μL of crude enzyme solution A into sample tubes.
   Control tube: Add 150 μL of crude enzyme solution B into control tubes.
4. Mix fully with the vortex mixer, incubate accurately at 37℃ for 3 min, incubate at 100℃ water bath for 5 min immediately. Then cool the tubes to room temperature with running water.

5. Take 320 μL into the microplate and measure the OD value of each well at 410 nm (the OD value of the sample well is record as A_1, the OD value of the control well is record as A_2, △ $A = A_1 - A_2$).

▲Summary operation table

	Sample tube	Control tube
Reagent 2 (μL)	600	600
Reagent 3 (μL)	150	150
Crude enzyme solution A (μL)	150	
Crude enzyme solution B (μL)		150
Mx fully, incubate accurately at 37℃ for 3 min, incubate at 100℃ water bath for 5 min immediately. Then cool the tubes to room temperature with running water. Take 320 μL into the microplate and measure the OD value of each well at 410 nm.		

▲Calculation

Definition: 0.01 OD value changed at 410 nm by 1 mg of tissue protein sample per minute in the reaction system at 37℃ that is defined as an enzyme activity

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

Note:

ΔA: △ $A = A_1 - A_2$

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

T: Reaction time, 3 min;

C_{pr}: The concentration of protein in sample, mgprot/mL.

f: The dilution factor of sample before tested.

Appendix I Data

▲Example analysis

For chinese yam tissue, take 0.1 g of chinese yam tissue, add 0.9 mL of reagent 1 , then homogenize the sample in ice water bath, centrifuge at 10000 g for 10 min at 4℃ , then take 0.15 mL of chinese yam tissue supernatant and carry the assay according to the operation table. 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, △ $A = A_1 - A_2 = 0.320 - 0.189 = 0.131$, the concentration of protein in sample is 1.97 mgprot/mL, and the calculation result is:

$$\text{PPO activity (U/mgprot)} = \frac{222.2 \times 0.131}{1.97} = 14.78 \text{ U/mgprot}$$