（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K227-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（410-430 nm）

Detection range：0.01-100 U/mL

Elabscience® Peroxidase (POD) Activity Assay Kit

(Plant Samples)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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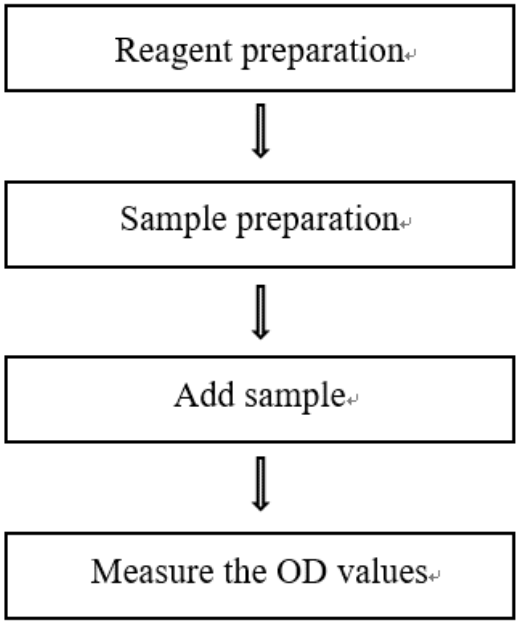
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	30 mL ×1 vial	60 mL ×1 vial	2-8℃, 12 months
Reagent 2	Chromogenic Agent	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months shading light
Reagent 3	Substrate Solution	1 mL ×1 vial	1 mL ×1 vial	2-8℃, 12 months
Reagent 4	Stop Solution	10 mL ×1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

① The 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 ℃ for 1 month protected from light.

② The 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 on spot. Store at 2-8 ℃ for 7 days.

③ The 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 on spot. Store at 2-8 ℃ for 7 days.

Sample preparation

① Sample preparation

Tissue sample:

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 ℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M，E-BC-K168-S).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

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

③ Add 200 μL of stop application solution into each tube, mix well 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.

Calculation

The sample:

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

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

[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 min.

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

f: dilution factor of sample before tested.

1000*: 1000 μg=1 mg.

12*: absorption coefficient.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

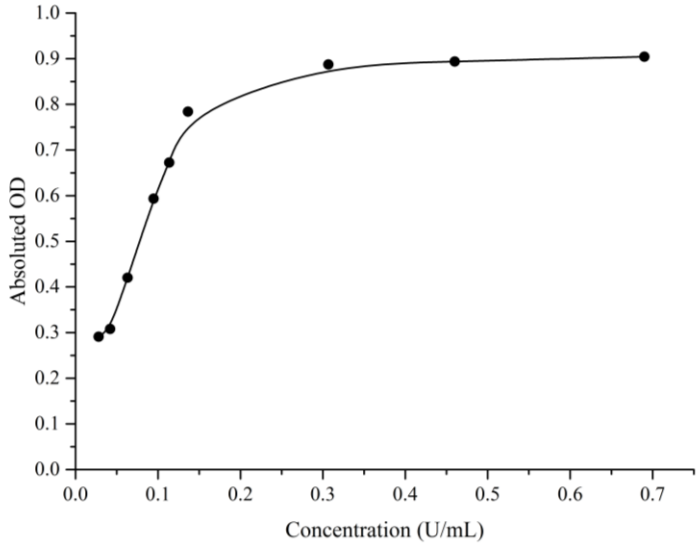
	Sample 1	Sample 2	Sample 3
Expected Conc. （U/mL）	12.5	47.6	88.5
Observed Conc. （U/mL）	12.4	50.0	90.3
recovery rate(%)	99	105	102

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.

2. 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), so the standard curve and data are provided as below for reference only：



Appendix II Example Analysis

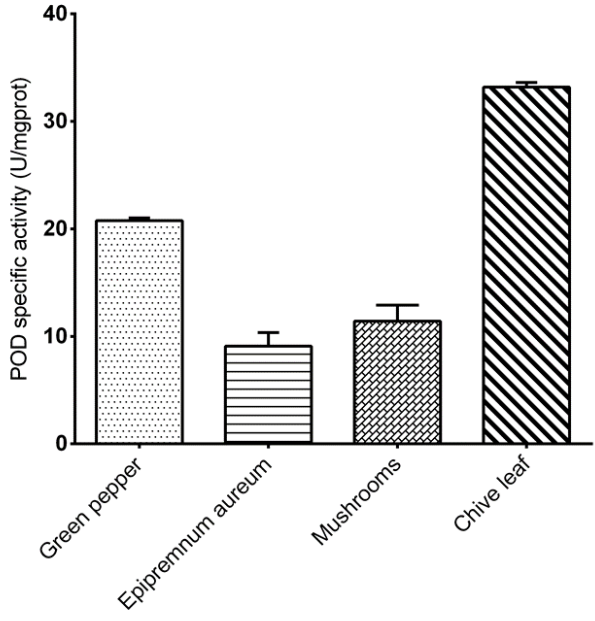
Example analysis:

Take 20 μL of 10% green pepper tissue homogenate supernatant carry 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 mgprot/mL, and the calculation result is:

$$\text{POD activity (U/mgprot)} = 0.406 \div (12 \times 1) \times 0.8 \div 0.02 \div 30 \div (2.18 \div 1) \times 1000$$
$$= 20.69 \text{ U/mgprot}$$

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.