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

Catalog No：E-BC-K227-S

Specification：50 Assays(25 samples)/100 Assays (50 samples)

Measuring instrument：Spectrophotometer（240 nm, 420 nm）

Detection range：0.5-40 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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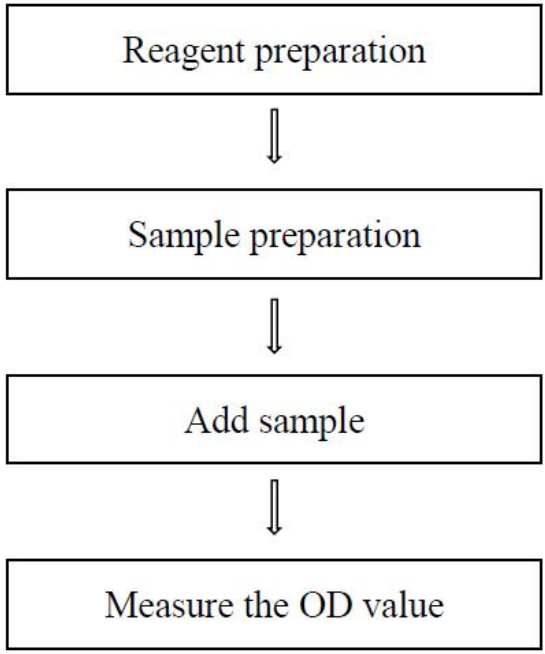
Assay summary



Intended use

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

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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	60 mL × 4 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 3	Substrate Solution	1.5 mL × 2 vials	1.5 mL × 4 vials	2-8°C, 12 months
Reagent 4	Stop Solution	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (240 nm, 420nm), 37°C incubator, Vortex mixer, Micropipettor, Centrifuge

Reagents:

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

Reagent preparation

① The preparation of chromogenic application solution：
Dilute one vial of chromogenic agent with 17.5 mL of double distilled water, mix well. Store at 2-8°C protected from light.

② The preparation of substrate application solution：
For each well, prepare 200 μL of substrate application solution (mix well 8 μL of substrate solution and 192 μL of double distilled water). 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 OD value is too low, add appropriate substrate solution.

③ The preparation of stop application solution：
For each well, prepare 1000 μL of stop application solution (mix well 500 μL of stop solution and 500 μL of double distilled water).

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°C.

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% 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). 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 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

Operating steps

① Sample tube: add 2.4 mL of buffer solution, 0.3 mL of chromogenic application solution, 0.2 mL of substrate application solution and 0.1 mL of sample into a 5 mL EP tube.

Control tube: add 2.4 mL of buffer solution, 0.3 mL of chromogenic 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 at 37°C for 30 min accurately.

③ Add 1.0 mL of stop application solution into each tube, mix well and centrifuge at 2300×g for 10 min.

④ Take the supernatant for detect the OD value. 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).

Calculation

The sample:

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

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

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

1: The optical path of cuvette, 1 cm.

V_1: Total volume of reaction solution, 4 mL.

V_2: The volume of sample in 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 tested.

*: Constant.

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)	6.20	18.40	30.50
%CV	4.1	3.7	3.6

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)	6.20	18.40	30.50
%CV	5.2	5.6	5.7

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	12.4	21.5	35
Observed Conc. (U/mL)	12.3	21.7	32.9
recovery rate (%)	99	101	94

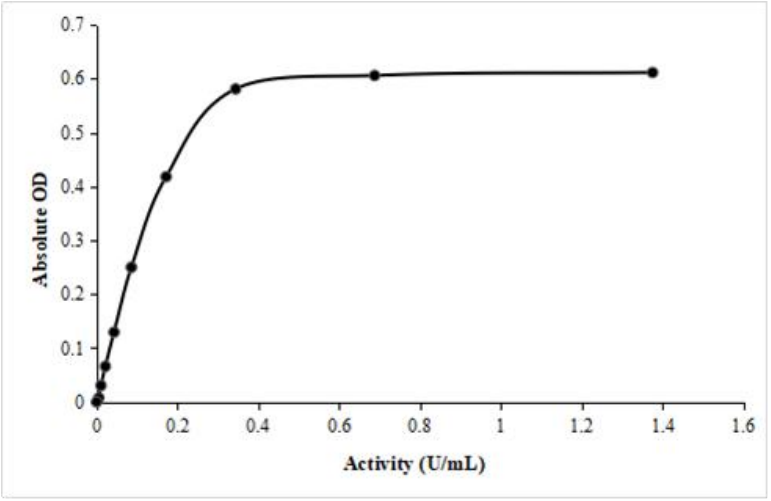
Sensitivity

The analytical sensitivity of the assay is 0.5 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:

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:

Activity (U/mL)	**1.375**	**0.69**	**0.344**	**0.172**	**0.086**	**0.043**	**0.022**	**0.011**	**0.005**	**0**
Average OD	0.655	0.649	0.624	0.461	0.293	0.173	0.109	0.074	0.051	0.043
Absoluted OD	0.612	0.649	0.581	0.461	0.250	0.130	0.109	0.031	0.008	0



Appendix II Example Analysis

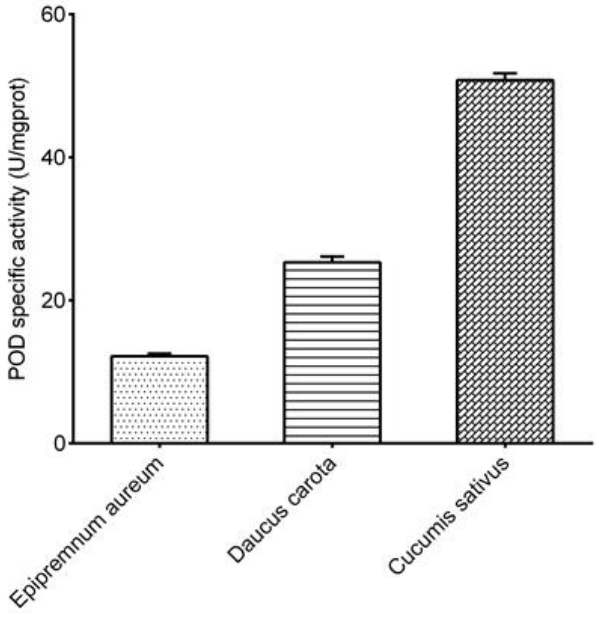
Example analysis:

For daucus carota tissue, take 0.1 mL of daucus carota tissue supernatant, carry the assay according to the operation steps. The results are as follows:

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

$$\text{POD activity (U/mgprot)} = \frac{0.263 - 0.088}{12 \times 1} \times \frac{4}{0.1} \div 30 \div 0.77 \times 1000 \times 1 = 25.25 \text{ U/mgprot}$$

Detect epipremnum aureum (the concentration of protein in 10% tissue supernatant is 1.87 mg/mL, dilute for 2 times), daucus carota (the concentration of protein in 10% tissue supernatant is 0.77 mg/mL), cucumis sativus (the concentration of protein in 10% tissue supernatant is 0.91 mg/mL, dilute for 2 times) according to the protocol, the result is as follows:



Appendix III Publications

1. Li L, Wang C, Wang W, et al. Uncovering the mechanisms of how corn steep liquor and microbial communities minimize cadmium translocation in Chinese cabbage[J]. Environmental Science and Pollution Research, 2024, 31(15): 22576-22587.
2. Alsamadany H, Abdulbaki A S, Alzahrani Y. Unravelling drought and salinity stress responses in barley genotypes: physiological, biochemical, and molecular insights[J]. Frontiers in Plant Science, 2024, 15: 1417021.
3. Alsamadany H. Physiological, biochemical and molecular evaluation of mungbean genotypes for agronomical yield under drought and salinity stresses in the presence of humic acid[J]. Saudi Journal of Biological Sciences, 2022, 29(9): 103385.
4. Shcherban A B, Skolotneva E S, Fedyaeva A V, et al. Effect of Biopesticide Novochizol on Development of Stem Rust Puccinia graminis f. sp. tritici in Wheat, T. aestivum L[J]. Plants, 2024, 13(23): 3455.
5. Otie V, Udo I, Shao Y, et al. Salinity effects on morpho-physiological and yield traits of soybean (Glycine max L.) as mediated by foliar spray with brassinolide[J]. Plants, 2021, 10(3): 541.
6. Li J, Chen S, Zhong J, et al. Removal of formaldehyde from indoor air by potted Sansevieria trifasciata plants: dynamic influence of physiological traits on the process[J]. Environmental Science and Pollution Research, 2024, 31(54): 62983-62996.

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.