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

Catalog No：E-BC-F012

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate reader

(Ex/Em=535/587 nm)

Detection range：0.0032-3 U/L

Elabscience® Peroxidase (POD) Activity Fluorometric Assay Kit

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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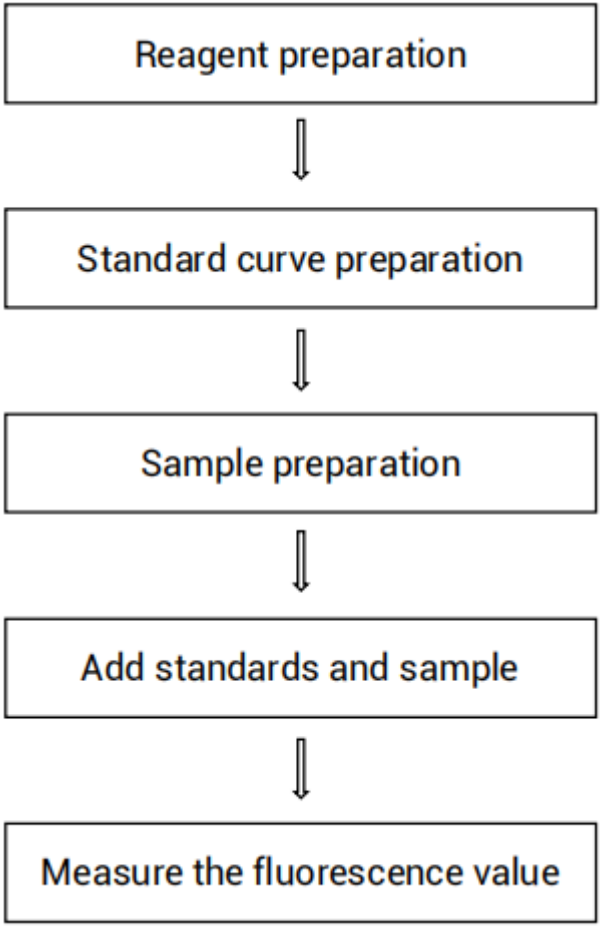
Assay summary



Intended use

This kit can be used to detect the peroxidase (POD) activity in tissue, cells plasma and serum samples.

Detection principle

Peroxidase (POD) is a class of oxidoreductases widely distributed in animals, plants, and microorganisms. It catalyzes the oxidation of various substrates by hydrogen peroxide (H_2O_2) or organic peroxides, while acting as an electron acceptor.

POD catalyzes the substrate to produce a fluorescent product, and POD activity is calculated by measuring the change in fluorescence intensity of the product at an excitation wavelength of 535 nm and an emission wavelength of 587 nm per unit time.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	14 mL × 1 vial	28 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	0.014 mL × 1 vial	0.028 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Chromogenic Agent	0.55 mL × 1 vial	1.1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	3 mmol/L Standard Solution	0.1 mL × 1 vial	0.2 mL × 2 vials	-20°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, 1×PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of Substrate Working Solution：
Before testing, please prepare sufficient Substrate Working Solution according to the test wells. For example, prepare 1000 μL of Substrate Working Solution (mix well 998 μL of double distilled water and 2 μL of Substrate). The substrate working solution should be prepared on spot protected from light and used up within 8 hours.

③ The preparation of Working Solution：
Before testing, please prepare sufficient Working Solution according to the test wells. For example, prepare 2200 μL of Substrate Working Solution (mix well 1995 μL of Buffer Solution and 5 μL of Substrate Working Solution and 200 μL of Chromogenic Agent). The Working Solution should be prepared on spot protected from light and used up within 1 hour.

④ The preparation of 20 μmol/L Standaed Solution：

Before testing, please prepare sufficient 20 μmol/L Standaed Solution according to the test wells. For example, prepare 150 μL of 20 μmol/L Standard Solution (mix well 149 μL of Buffer Solution and 1 μL of 3 mmol/L Standard Solution). Store at -20°C for 7 days protected from light.

⑤ Standard curve preparation：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 20 μmol/L Standard Solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 4, 8, 10, 12, 14, 16, 20 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**4**	**8**	**10**	**12**	**14**	**16**	**20**
20 μmol/L Standard (μL)	0	40	60	80	100	140	160	200
Double distilled water (μL)	200	160	140	120	100	60	40	0

Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ˚C for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

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 min at 4˚C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4˚C.

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

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Dilution of sample

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

Sample type	Dilution factor
10% Porcine liver tissue homogenization	6-10
10% Mouse liver tissue homogenization	6-10
10% Mouse spleen tissue homogenization	1
10% Chinese yam tissue homogenization	3-8
10% Chili pepper flesh tissue homogenization	8-15
10% Orange peel tissue homogenization	1
10% Apple flesh tissue homogenization	1
Rat serum	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.

Operating steps

① Add 180 μL of Buffer Solution to each well.

② Standard well: Add 20 μL of Standard Solution with different concentrations to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

③ Mix fully with microplate reader for 5 s. Measure the initial fluorescence value (F_1) of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm with fluorescence microplate reader.(The standard curve is fitted to the standard well in F_1 value).

④ Incubate at 37°C for 10 min. Measure the fluorescence value (F_2) of each well.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the Corrected fluorescence value.

3. Plot the standard curve by using Corrected fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue or cell sample:

Definition: One unit of enzyme is defined as the amount enzyme in 1 g protein will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\frac{\text{POD activity}}{\text{(U/gprot)}} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

Serum and plasma sample

Definition: One unit of enzyme is defined as the amount enzyme in 1 L plasma or serum will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\frac{\text{POD activity}}{\text{(U/L)}} = (\Delta F - b) \div a \div T \times f$$

[Note]

ΔF: The fluorescence value of the sample well changes, $F_2 - F_1$.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: Reaction time, 10 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% Mouse liver tissue homogenization samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Three 10% Mouse spleen tissue homogenization samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/gprot)	0.31	0.35	0.41
%CV	1.2	2.0	1.2

Inter-assay Precision

Three 10% Mouse liver tissue homogenization samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Three 10% Mouse spleen tissue homogenization samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/gprot)	1.60	1.33	0.98
%CV	8.2	7.6	10.0

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/gprot)	0.280	0.350	0.420
Observed Conc. (U/gprot)	0.303	0.350	0.407
Recovery rate (%)	108	100	97

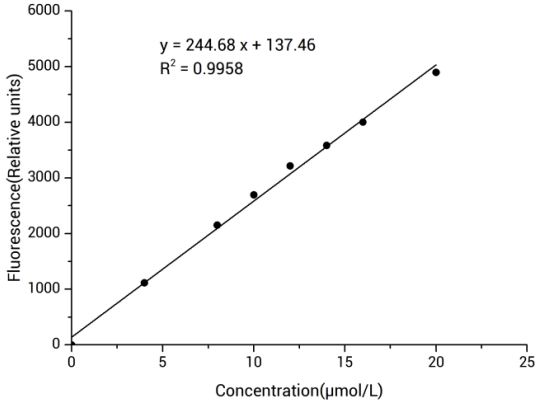
Sensitivity

The analytical sensitivity of the assay is 0.0032 U/L. 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 fluorescence 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：

Concentration (μmol/L)	0	4	8	10	12	14	16	20
Fluorescence value	1	1100	2088	2707	3158	3638	4003	4715
	0	1129	2212	2681	3270	3270	3999	5082
Average fluorescence value	1	1115	2150	2694	3214	3585	4001	4899
Corrected fluorescence value	0	1114	2149	2693	3213	3584	4000	4898



Appendix II Example Analysis

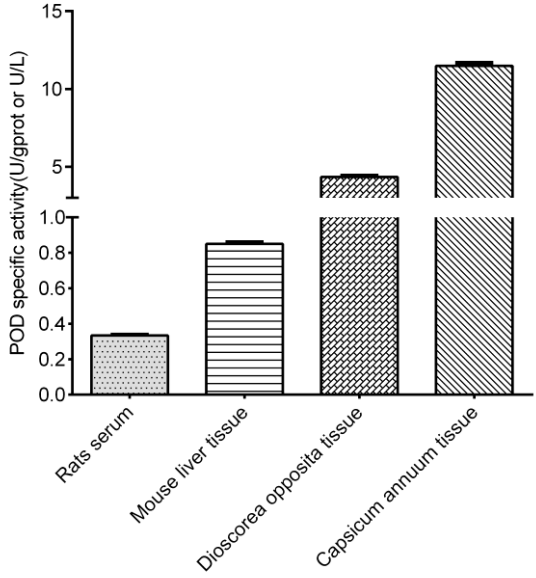
Example analysis：

Take 20 μL of the 9-fold diluted 10% Chili pepper flesh tissue homogenate supernatant to the well of black microplate. Proceed according to the operating steps. The results are as follows:

standard curve: y = 244.68 x + 137.46. The F_1 of sample is 181, the F_2 of sample is 3685, $\Delta F = F_2 - F_1 = 3685 - 181 = 3504$, the concentration of protein is 1.05 gprot/L, and the calculation result is:

POD activity(U/gprot) = (3504 - 137.46) ÷ 244.68 ÷ 10 × 9 ÷ 1.05 = 11.79 U/gprot

Detect Rat serum (dilute for 10 times), 10% mouse liver tissue homogenization (the concentration of protein is 12.31 gprot/L, dilute for 8 times), 10% chinese yam tissue homogenization (the concentration of protein is 1.05 gprot/L, dilute for 4 times) and 10% Chili pepper flesh tissue homogenization (the concentration of protein is 1.05 gprot/L, dilute for 9 times), 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.